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BETTER THINGS FOR BETTER LIVING

. . . *THROUGH CHEMISTRY*

DIRECT EVIDENCE FOR THE MULTIPLICATION OF ASTER-YELLOWS VIRUS IN ITS INSECT VECTOR[1]

Karl Maramorosch

Although transovarial passages (5, 6, 10, 11) of two plant viruses through the eggs of their vectors provided evidence of virus multiplication in these vectors, the evidence was considered inadequate by some workers (1). This stimulated a return to the problem. It seemed that the question of whether or not any given plant virus multiplied in its insect vector could easily be settled if the virus were carried in serial passages from insect to insect, with the inoculum adequately diluted at each passage. Such a test was made for the animal virus of equine encephalomyelitis by Merrill and TenBroeck (25), but no similar evidence was available for any plant virus. The discovery by Black (3) that aster-yellows virus could be transmitted by needle inoculations to its insect vector, the aster leafhopper *Macrosteles divisus* Uhler, made it possible to obtain such evidence for aster-yellows virus in its carrier insect. A preliminary report of work on the problem has already been presented (20). This paper describes in detail ten consecutive passages through the leafhopper in experiments started in March 1950, and carried out over a period of 13 months.

MATERIALS.—All experiments were carried out under controlled conditions of temperature, light, humidity, and wind velocity. Figure 1 represents a diagram of one of the temperature control chambers used. The

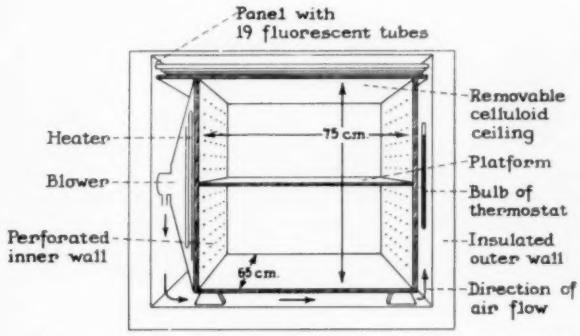

FIG. 1. Diagram of a temperature control chamber, with fluorescent lights (door removed).

inside dimensions were 65 × 75 × 75 cm. A removable ceiling made of 0.020-in. (0.5-mm.) transparent cellulose acetate was found to reduce the light intensity by only 2 per cent and at the same time to insulate the working part of the box sufficiently. Six holes, 0.5 cm. in diameter, were made in the celluloid to avoid excessive water vapor condensation on the ceiling. Two inner walls were perforated by 13 rows of holes, 0.5 cm. in diameter, 4 cm. apart. These holes served primarily for air flow, but were also used for the insertion of metal blocks which supported the platform at desired levels. Behind one of the perforated walls a heater of high-resistance wire was placed and covered by a metal box with a blower. The speed of this blower was regulated by a rheostat. The air flow was directed through the space below the proper inside box and back into it through the opposite wall, as indicated in the diagram. It proved desirable to cover the holes below the platform by pieces of cardboard. A gas bulb type thermostat (Minneapolis Honeywell) was placed behind the wall opposite to the heater. The boxes were kept in an air-conditioned room at 10° C. and the inside temperature maintained at 25° ± 0.5° C. Cool air from the room entered each box through holes in the outer walls and mixed continuously with the warm air inside. Fluorescent lamps were chosen as the source of light because of their low temperature, in spite of some disadvantages like low intensity and excessive weight of electric resistance (ballast) equipment, which could be partially or completely overcome. Each box was lighted by

FIG. 2. View of the insectary. (Photograph by J. A. Carlile.)

19 fluorescent tubes, mounted on a panel as close together as their widths made practical. The ballasts were placed outside the room so that their heat was not dissipated in the refrigerated area. By means of separate switches, each fluorescent tube could be connected with either one or two ballasts. By the use of double ballasts for single lights, the intensity was increased by approximately 60 per cent. With new tubes at a distance of 44 cm. (19 in.) from the plastic ceiling, the maximum intensity of light was 4000 foot-candles, whereas with single ballasts at the same distance it was 2500 foot-candles. The ballast arrange-

[1] Accepted for publication August 28, 1951.
Awarded an A. Cressy Morrison Prize in Natural Science in 1951 by the New York Academy of Sciences.

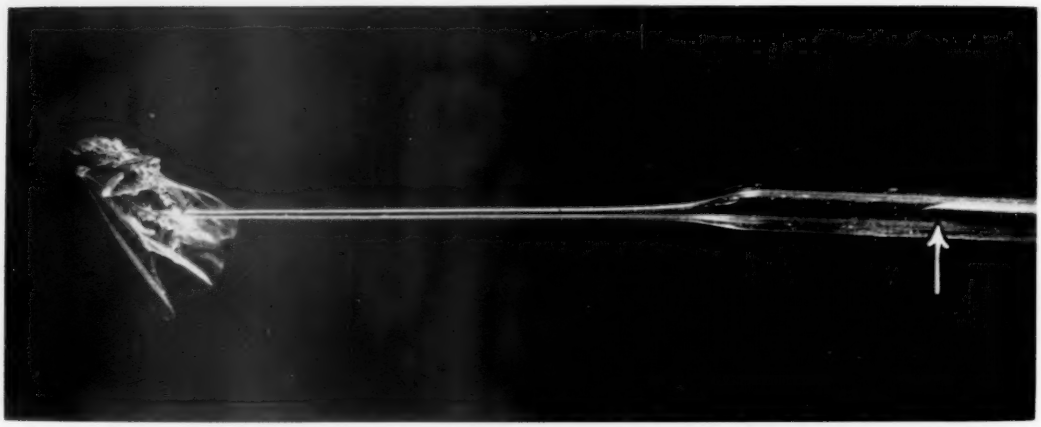

FIG. 3. Method used for injecting a leafhopper. Arrow points to the end of a hypodermic needle that was cemented into a Pyrex capillary pipette. (Photograph by J. A. Carlile.)

ments were suggested by Went's description of the Earhart Plant Research Laboratory in Pasadena (31). The lights were turned on for 16 hr. a day by a standard automatic electric clock.

China asters (*Callistephus chinensis* Nees), used as test plants, were grown from commercial seed in flats and transplanted to 2½-in. pots.

A stock colony of virus-free *Macrosteles divisus* Uhler was kept in an insectary (Fig. 2) on rye plants, which are immune from aster yellows.

A specially constructed microsyringe, calibrated to 1/8000 cc., described by McMaster and Hageboom (24), permitted an accurate measurement of the volume of inoculum introduced into each insect. Ordinary metal hypodermic needles of 23 to 26 gauge and Pyrex micropipettes cemented to them with a paste of litharge and glycerine were used (21) (Fig. 3). When a colony of insects was employed, the insects were macerated in a commercially available TenBroeck tissue grinder (29). Single insects were ground in a similar micromortar of small dimensions (8).

METHODS.—The viruliferous insects, which served as virus source, were obtained by feeding 100 virus-free leafhoppers from stock cultures containing approximately equal numbers of males and females on diseased aster plants for 2 weeks at 25° C. When tested individually for 2 days on asters, 83 of them proved infective. The 100 insects, weighing approximately 140 mgm., were crushed in the tissue grinder and 999.860 cc. of neutral buffered saline added at the time of maceration, to make approximately a 1:7000 dilution of the insect pulp. In subsequent passages the weight of the survivors was taken as the basis for making a 1:1000 dilution. Single insects weigh at least 10 times as much as the inoculum used for their injection (1.2 to 1.4 mg., as compared to the weight of 1/8000 cc. = 0.12 mg., approximately). Therefore the actual dilution of the original inoculum in each consecutive passage increased by a factor of 10^{-4}.

The diluted pulp was centrifuged at 3000 r.p.m. for 10 min. at 0° C. and the supernatant fluid was used for injections. The injections were performed under a fluorescent light and a magnifying glass in a cold-room at 0° C., which immobilized the insects. An attempt was made to insert the needle between the third and fourth segment of the abdomen, thus introducing the measured amount of 1/8000 cc. into the abdominal cavity. This procedure was a modification of leafhopper inoculations developed by Storey (27) and Black (3). The essential difference consisted of the introduction of a very small but accurately measured volume of inoculum. Figure 3 shows an insect during injection. The insects were pushed off the needle by means of an insect pin sealed in a glass rod; they were then slowly warmed to room temperature. Subsequently they were caged on rye plants (*Secale cereale* L.), and placed in chambers at 25° C. Females were employed for the injections because they were somewhat larger and therefore more easily injected. The size of the leafhoppers is illustrated in figure 4. Injected insects were transferred every 10 days to fresh rye plants and after 30 days the survivors were tested individually for 48 hr. on young aster plants. They were then macerated, diluted, and used for the next passage. The aster plants were kept under observation in a greenhouse for 6 weeks after the removal of the insects. Noninjected control insects from

FIG. 4. Male and female *Macrosteles divisus* Uhler. Note larger size of females. (Photograph by J. A. Carlile.)

TABLE 1.—*Serial passage of aster-yellows virus through the aster leafhopper*

Passage[a]	No. of injected insects	Days after injection on: Rye	Asters[b]	Survivors[c]	Calculated dilution
A.					
1	200	30	2	36 (8)	$10^{-3.8}$
2	300	30	2	16 (3)	10^{-8}
3	150	30	2	2 (0)	10^{-12}
4	300	30	2	34 (5)	10^{-16}
5	100	30	2	5 (0)	10^{-20}
6	200	30	2	22 (5)	10^{-24}
B.					
7	100	6	54*	8 (2)	10^{-28}
8	240	40	25**	12 (4)	10^{-32}
9	50	19	13*	16 (6)	10^{-36}
10	50	30	20	4 (1)	10^{-40}

[a]The diluted insect juice in the fourth passage was filtered through sintered glass.

Penicillin was added in the fourth, fifth, sixth, and seventh passages.

[b]Individual insects were transferred to individual aster plants: *three times weekly; **every 5 days.

[c]Number of infective survivors in parentheses. In series A, all 100 control insects for each passage survived, and in series B all 20 control insects for each passage survived.

the same stock culture were caged on aster plants and transferred on the same days as the injected leafhoppers.

EXPERIMENTS AND RESULTS.—*Serial passage.*—The results of infectivity tests of the first six passages were not known at the time of maceration of the survivors. These passages are summarized in table 1, A. One hundred viruliferous insects were used as a source of virus in the first transfer. The leafhopper pulp (140 mg.) was diluted 1:7000, so that the dilution of the virus in the insect juices actually was greater than stated. Parts of these diluted juices were injected into 200 virus-free leafhoppers which were maintained for the succeeding 30 days on rye plants at 25° C. The 36 insects that survived were tested individually for 48 hr. on young aster plants. After 3 weeks this test showed that eight hoppers were infective. In the meantime the 36 insects were used as a source of virus for the next passage. Three hundred insects were injected; 16 survived 30 days and were used for the third passage. Three of them were later found to have been infective. In the third passage, 150 insects were injected and only two survived. These two survivors were macerated and, in an attempt to reduce the high mortality rate, penicillin was added and the supernatant filtered through a coarse glass filter. Of 300 injected insects in this passage, 34 survived and five proved infective. Thus the virus was not lost in the third passage in spite of the negative infectivity test. Penicillin was also added in the next three passages. In the fifth passage five insects survived but none of these proved infective; in the sixth passage, five of 22 survivors transmitted the virus.

The following four passages (Table 1, B) were made with some modifications of the previously used method. The 22 survivors of the sixth passage were macerated with the addition of penicillin, and 100 virus-free insects were injected. Eight survived a period of 60 days and two of them were infective. The eight survivors served as a source for the injection of 240 insects, of which 12 survived 65 days and four were infective. The insects of these two passages were not kept on rye continuously, but after a 6-day and a 19-day period, respectively, they were caged on asters and transferred three times weekly. Only the four infective survivors of the eighth passage were used for the inoculum of the ninth group. The injected insects were transferred three times weekly to fresh asters. After 32 days, 16 of the 50 injected leafhoppers survived and four of them proved infective. The tenth and last passage, with 50 injected leafhoppers, was kept 30 days on rye, then tested on aster plants, and of four survivors one proved to be infective. The estimated dilution used to inoculate the insects of the tenth passage in the series, if no multiplication had occurred, would be at least 10^{-40}, but aster-yellows virus in insect juices has been found infective at dilutions of 10^{-3} but not 10^{-4} or higher.

In experiments which will be discussed in more detail elsewhere (23), the concentration of virus in the first and ninth passages was measured by the use of a new method. The tests showed no decrease in virus concentration of the inoculum used in the ninth passage over that used in the first, and provided further direct evidence for multiplication of aster-yellows virus in the insect host.

Transfers of virus from "noninfective" viruliferous insects.—It was found, in two instances, that virus was carried over to the next group of insects although infectivity tests of the source insects proved negative. This occurred in the third and fifth passages. It is possible that some insects that did not transmit virus during the 48-hr. test feeding period were nevertheless infective, or that the incubation period of the virus in some insects was not completed by the time of their maceration. An attempt was therefore made to find whether or not very long incubation periods may account for the negative infectivity tests. The insects of the seventh passage were kept for 54 days on test plants, and not, as in the first six passages, for but 2 days. They were transferred to fresh plants three times weekly. The survivors were macerated individually and diluted to 10^{-3} and groups of 30 insects injected with each dilution. Insects No. 5 and No. 30 were infective in the seventh passage. The virus from No. 30 passed to two injected insects, whereas none of the insects injected with juice of No. 5 became infective. But the noninfective No. 21 of the seventh passage also contained enough virus to render two insects of the eighth passage infective. This may mean that in insect No. 21 the incubation period of the virus was not completed in the long span of 60 days which had elapsed since it was injected. This appears likely because in insect No. 5 of the seventh group the required incubation period was shown to be 52 days. It should be mentioned that in other tests of insects that acquired the virus by feeding, the average

incubation period at 25° C. was 12 days. It was shown by Black (4) that aster-yellows virus can be recovered and transferred from viruliferous insects a number of days before the incubation is completed.[2]

Control experiments.—The control insects, amounting to 100 for each of the first six passages, came from the same stock as the injected ones and all proved virus-free. During winter months, when the last four passages were made, and outside contamination was unlikely to occur, the number of control insects was reduced to 20 for each passage. This was considered as a sufficient number because the stock of leafhoppers lived and bred entirely on rye plants, and the possibility of contamination of the stock culture in the insectary was therefore practically eliminated. In the seventh, eighth, and ninth passages insects were transferred to aster plants three times weekly by means of "leaf cages" (22). Individual hoppers were confined to single cages that were fastened to leaves. From five to ten control plants were inoculated at each transfer and all remained healthy.

To confirm the immunity of rye from aster-yellows, established long ago by Kunkel in early experiments with the disease (12), 50 viruliferous females were caged on rye and kept on it until some nymphs of the next generation reached the third instar stage. The 100 largest nymphs were caged individually on asters at 25° C. for 4 weeks. None of them transmitted aster yellows.

In a preliminary test it was found that at 25° C. approximately 47 per cent of insects acquired aster-yellows virus in a one-day infection feeding. It seemed desirable to define more accurately the length of time during which insects could be kept, without acquiring the virus, on inoculated test plants in which the incubation period was not completed. Forty aster plants were inoculated by means of infective insects, five feeding on each plant. Together with these infective adult insects, single virus-free nymphs were caged on the plants. After 24 hr. the infective adults and the test nymphs were removed, the adults discarded, and the nymphs caged on rye. Forty virus-free female leafhoppers were caged on the plants for 24 hr., then removed to rye and replaced by another group of 40 virus-free females. This was repeated until, on the 9th day, the first plant in the group showed symptoms of disease. The 40 insects which were removed on each day and kept at 25° C. on rye for 21 following days, were then tested on asters for one week. Of 312 survivors, one picked up virus on the 7th day, that is, 2 days before the first disease symptoms were observed. On the 8th day an additional nine insects acquired aster-yellows virus. Of the 40 inoculated plants all but three eventually became diseased. Ninety control insects, tested on asters, proved virus-free. The test showed that at 25° C. under the conditions of the experiment none of the tested insects were able to acquire the virus during the first 6 days of the incubation period of the virus in the plant. Therefore it was also concluded that the 2-day test feeding after each of the first six passages, as well as the transfers on asters in the later passages which were made every 2 to 5 days, could not have accounted for any increase of the virus in the tested insects.

Mortality of injected insects.—The high mortality of injected insects during the second and subsequent transfers presented a serious problem to the continuation of the serial passage experiment. It actually may have accounted for Black's failure to succeed with similar experiments in 1939-1942.[3] It was known from earlier tests by Black that the mortality could be decreased by filtration, but filtration also reduced the amount of virus in the inoculum.[4] Aster-yellows virus is presumably large and can be filtered only with difficulty. A preliminary test was made to find whether or not the mortality was due to an increase in pathogenicity of the virus towards the vector. This hypothesis could not be excluded in view of the fact that the serial passage eliminated the alternating plant host entirely from the life cycle of the virus. Ordinarily, when the virus alternates between plant and insect vector, no deleterious effect on the vector can be observed, although it would be difficult to detect any small disturbances or even small differences in the percentage that died. An alternative hypothesis could explain the increased mortality as due to bacterial and fungus contamination in injected insects. Undoubtedly many injected insects die of septicemia, and the mechanical injury even without infection may be responsible for some deaths.

An attempt was made to compare the mortality rate of insects injected with virus-containing and virus-free insect juices. Fifty virus-free females were injected with a 1:100 dilution of juices from infective leafhoppers, and another group of 50 virus-free females of the same age injected with a 1:100 dilution of juices from virus-free hoppers. Both groups of injected insects were transferred five times during 30 days to fresh rye plants and kept continuously at 25° C. under identical controlled conditions. The death rate in the two groups was almost exactly the same. On the 31st day the 10 and 12 survivors, respectively, of the viruliferous and virus-free leafhoppers were macerated and diluted 1:100, and two groups of 50 virus-free females were injected with the dilutions. The injections were carried out with a single needle, the virus-free dilution being injected first. Utmost care was exercised to prevent excessive wounding. The 1/8000 cc. of inoculum was measured in the same way as in the serial passages. A pronounced decrease in the survival of the virus-carrying insects was observed during the first 3 days following injection (from 50 to 24, as compared to 50/42 in the virus-free group), and after 9 days the number decreased to 13, as compared to 32 in the second group. But on the 23rd day the

[2] Transfer from such insects can be described as "blind." This term is used in experimental transmission of animal viruses for the method whereby transfer to a healthy animal is made from an inoculated one at a time when no signs of infection can be noted.

[3] Personal communication.

[4] Personal communication.

number of survivors in both groups reached the same level (12 and 13, respectively). Noninjected insects decreased very slightly in number, due to death, and of 50 control insects in the two tests survivors numbered 40 and 37, respectively, by the 30th day of the experiments.

Dilution end-point.—It seemed desirable to find the dilution end-point of aster-yellows virus from insect juice in view of the fact that evidence for multiplication was derived from the calculated "dilution" in the serial passage. From earlier experiments by Black (4) it was known that a dilution of 10^{-3}, but not 10^{-4}, will render injected insects infective.

Several tests were made and a more detailed account will be given elsewhere in a study of virus concentration as related to the length of incubation periods in inoculated insects. Only one of the experiments will be briefly mentioned. Groups of 30, 45, 50, and 40 insects, respectively, were injected with dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} of juices from virus-containing leafhoppers. The injected insects were transferred on every day to individual fresh aster plants in colonies of not more than three, for 48 days, and afterwards three times weekly until the 64th day, when the last insect died. Transmission was obtained in the groups injected with 10^{-1}, 10^{-2}, and 10^{-3} dilutions. No transmission occurred with a 10^{-4} dilution. In two other tests negative results with 10^{-4} dilutions and transmission with 10^{-3} also confirmed Black's finding.

The positive infectivity test in the first transfer of the serial passage experiment occurred within 32 days, as shown by the eight insects that were rendered infective (Table 1). It seems very likely that the dilution of the inoculum used, $10^{-3.8}$, was near the dilution end-point. It is still possible, however, that occasionally an insect would become infective following an injection of a somewhat higher dilution.

Discussion.—Earlier evidence indicated that several viruses multiply in both plants and insects. Experimental evidence was presented for the multiplication of rice stunt (10, 11), aster-yellows (4, 13, 14, 15, 19), club-leaf (5, 6), and wound-tumor virus (7, 18). These viruses have three characteristic features in common: the high specificity of vectors, the retention of the virus, and the incubation period. These features had led long ago to the assumption that such plant viruses might possibly multiply in their vectors. The opposite view was expressed in the past 15 years by a few workers (1, 2, 9, 26, 28, 30).

The aster-yellows virus was carried in 10 serial passages in the aster leafhopper. If the virus had been diluted without multiplication, the final dilution used to inoculate the tenth group of the series would have been 10^{-40}. This, of course, is inconceivable for a number of reasons. The virus of aster-yellows derived from viruliferous insects was shown to have a dilution end-point below 10^{-4}. No virus is known to have a dilution end-point[5] above 10^{-10}. In a recent paper Black pointed out that a mass of hydrogen weighing the same as a female leafhopper (1.7 mgm.) contains only about 5.1×10^{20} molecules (6). Therefore, the serial passage provided adequate direct evidence for multiplication of the aster-yellows virus in the aster leafhopper. Further evidence was provided when the concentrations of virus in the first and ninth transfers were compared and it was found that no decrease occurred in the virus concentration during the nine passages.

It should be pointed out that leafhoppers are sucking insects and do not take up living plant cells. Since viruses multiply only inside of living cells, the multiplication must have occurred in cells of the insect.

A few of the implications of plant virus multiplication in insects have recently been pointed out by Black (6). The virus of aster-yellows can no longer be considered as solely a plant virus. It is really both a plant and an animal virus and may therefore serve as a connecting link between the two groups. This will have to be considered in any future classification of viruses, heretofore separated into animal, plant, and bacterial groups. It allows also some speculation as to the origin of the yellows group of plant viruses. They could have originated as insect or animal viruses. Some of them have the capacity of passing through the egg of the vector to the progeny and are thus maintained indefinitely in insects without the necessity of alternating plant hosts (6, 11). An adaptation permitting them to multiply in plants may possibly have come about as a further step in evolution, and the passage through the egg may then have been gradually lost.

The ability of some viruses to multiply in such diversified hosts as plants and animals suggests strongly that they are living organisms. The early hypothesis of a precursor responsible for virus multiplication, which even in recent years received some support, can hardly account for aster-yellows multiplication. It seems unlikely that a large molecule-precursor would occur in two hosts that are so unrelated.

The high specificity of yellows viruses in relation to their insect vectors can easily be explained on the basis of multiplication. The specificity also supports the hypothesis of the origin of these viruses in insects. The aster yellows virus is certainly better adapted to its vector, in which it causes no visible harm, than to plant hosts in which it invariably causes disease symptoms and sometimes death.

The possible harmful influence of the virus on the vector after it was passed from insect to insect has been pointed out. Although preliminary results had first suggested such deleterious effect, closer analysis does not permit such a conclusion at present.

The "blind" passage of virus was most likely due to a very long incubation period in some injected insects. On the other hand, it is possible that some in-

[5] The highest dilution end-points of 10^{-10}, of viruses derived from tissues, without concentration, were found for Russian Far East Encephalitis, Venezuelan Equine Encephalitis, Western Equine Encephalitis, and Eastern Equine Encephalitis (Olitsky, Peter K., personal communication), also for St. Louis Encephalitis (16) and yellow fever (17). Tobacco-mosaic virus in tobacco plant juices has a dilution endpoint of 10^{-7} (Kunkel, L. O., personal communication).

jected insects harbored virus but never became infective because the virus did not reach the saliva. It is hoped that further investigations will elucidate this possibility.

The finding that the incubation period varied markedly in the vector, in relation to the dose, may indicate an essential difference between the mechanism of infection of plants and animals. Wounding may be a necessary condition for the infection of plants but not of animals.

The basic study of yellows viruses promises to be of importance to animal workers as well as plant workers.

SUMMARY

The virus of aster yellows was carried in the vector *Macrosteles divisus* through 10 serial passages. The transfer from insect to insect was made by means of injections, of measured volumes of 1/8000 cc. per insect, into the abdominal cavity. The insect pulp was diluted with neutral buffered saline to 10^{-3} of the weight of the surviving leafhoppers. In the first six passages each group of injected hoppers was kept for 30 days, following injection, on rye plants immune from aster yellows, then tested 48 hr. on aster plants and used for the next transfer. In the later passages insects were tested on asters and transferred three times weekly to a fresh set of plants for periods up to 60 days. Direct evidence was provided for the multiplication of the virus in the insect vector, as some insects of all but the third and fifth groups were found infective. The estimated dilution used to inoculate the tenth group of the series, if no multiplication had occurred, would have been 10^{-40}, but the dilution endpoint of the virus from macerated insects was found to be below 10^{-4}. A comparison of virus concentration in the inoculum of the first and ninth passages showed no decrease in concentration. It is concluded that the aster-yellows virus, multiplying in both its plant and insect hosts, can be considered an animal as well as a plant virus.

THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, NEW YORK

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GLADIOLUS AS A VIRUS RESERVOIR[1]

G. H. Bridgmon and J. C. Walker

During the summers of 1948 and 1949 a destructive disease occurred in several garden plantings of bean (*Phaseolus vulgaris* L.) in the vicinity of Madison, Wisconsin. Severe top necrosis, stunting, and killing were noted on many varieties. The symptoms suggested a severe type of yellow bean mosaic (bean virus 2), but no traces of sweet clover, the common overwintering host of this virus in this region, could be found. Since gladiolus was found by McWhorter et al. (14) to be a carrier of bean virus 2, near-by gladiolus plantings, and those of local commercial growers, were examined for possible sources of viruses infectious to bean.

A large number of mechanical inoculations were made to bean from gladiolus, and the yellow bean mosaic virus was recovered in many instances. In addition, extreme necrosis too severe to be typical of the yellow bean mosaic was noted in many cases, although McWhorter did report severe necrosis of bean incited by a strain of the yellow mosaic virus. Because bean may be infected by other viruses, e.g. tobacco virus 1 (16, 18), tobacco ringspot virus (11, 17), and a strain of cucumber virus 1 (23), mechanical inoculations from gladiolus were made to healthy tobacco, *N. tabacum* L., which is not susceptible to bean virus 2. These inoculations gave a few typical ringspotlike local lesions and often a mottle not unlike that incited by tobacco virus 1 and cucumber virus 1. It soon became apparent that there were two previously unreported viruses present in gladiolus, one a strain of cucumber virus 1 and the other a strain of the tobacco ringspot virus. These viruses were always recovered from gladiolus plants showing typical white break mosaic symptoms as described by Nelson (15) and Dosdall (9).

This investigation was undertaken to identify the unknown viruses and thus contribute to the solution of the white-break disease problem of growers, as well as to clarify the bean-gladiolus relationship in the home garden. A preliminary report has been published (6), and confirmatory evidence has been reported from Canada by Berkeley (3).

METHODS AND MATERIALS.—The greenhouses used were frequently sprayed and fumigated to control aphids and other insects. Air temperatures were maintained between 24° and 28° C., except where otherwise stated. Cultures were obtained from the plantings of commercial gladiolus growers in the vicinity of Madison, Wisconsin. The final characterization of each type of virus found was made on an isolation from a single gladiolus plant, except where otherwise indicated. Separations were based on symptoms and host range. A typical strain of cucumber virus 1 was obtained from James Johnson; a green ringspot strain of the tobacco ringspot virus from R. W. Fulton; and a yellow ringspot strain of the same virus from G. S. Pound. Stock cultures and those isolated from the field were maintained on *N. tabacum* L. and *N. glutinosa* L. or on the F_1 hybrid of the two species. Isolations of bean virus 2 were maintained on Idaho Refugee, a variety highly resistant to bean viruses 1 and 4.

Young greenhouse plants were sprinkled with fine carborundum powder and mechanically inoculated, then thoroughly rinsed with distilled water. Except as indicated, a minimum of 10 plants was used for determining symptoms and host range and 10 plants from the same source were used as controls. Wherever symptoms did not occur on the inoculated hosts, the presence or absence of the virus was determined by subinoculations to healthy indicator plants.

ISOLATIONS FROM GLADIOLUS.—Isolations were attempted from both apparently healthy and diseased gladiolus plants. Two types of symptoms on gladiolus foliage were noted: 1) a faint mottle, and 2) the type previously described in connection with white break (9, 15). The symptoms were confined to varieties with colored petals, except for one plant in a white variety which had foliage typical of white break and a single

Fig. 1. White break of gladiolus. A, foliage symptoms. B, malformation of blossoms.

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blossom suggestive of the disease. White break was characterized by gray or yellowish-green streaks and spots on the foliage, entire leaves usually being affected (Fig. 1, A). Flower symptoms, not nearly so uniform as those on the leaves, consisted of broken areas on the petals, which were lighter than normal in color and contained spots or streaks surrounded by gray or gray-green borders. Florets were often reduced in size or twisted and so malformed that they did not open completely (Fig. 1, B). Plants affected with white break blossomed later than normal. The percentage of affected blossoms increased as the season progressed, as high as 50 per cent of the plants being affected. Break was noted in many varieties and the incidence was particularly high in Valeria, Picardy, Ethel Cave Cole, and Burma.

Isolations from plants having no foliage or floral symptoms were usually unsuccessful, but bean virus 2 was recovered from one such plant. Two isolations from plants with badly broken blossoms were negative. In two attempts in which only the youngest leaves had symptoms, a virus could not be isolated. Isolations from some of the floral parts were negative regardless of extreme symptoms of break. All attempts to isolate the viruses from dormant corms, produced by plants having symptoms of white break the previous year, were unsuccessful. However, isolations were frequently successful from the first leaves arising from the corms, even from those of unplanted corms.

Isolation of bean virus 2.—The usual procedure in isolating from diseased gladiolus plants was to inoculate plants of Idaho Refugee bean and tobacco. This bean responds to infection by bean virus 2 by developing mottle. It is not infected by most strains of cucumber virus 1 but responds to one strain in the form of mottle and necrosis (23). Its response to tobacco ringspot virus is necrosis of young leaves and stems. When infection of bean occurred, the virus was inoculated to tobacco, white sweet clover, black cowpea, and additional bean varieties to determine with certainty whether or not bean virus 2 was concerned.

Bean virus 2 was isolated in nine of 30 isolations attempted. In most of these the isolates produced symptoms on Idaho Refugee bean which corresponded with those of the typical strain. In three cases, however, pod distortion occurred on inoculated plants. To investigate further the possibility of the pod-distorting strain overwintering in gladiolus, mechanical inoculations were made to varieties which serve to distinguish between this strain and the typical strain. Inoculations to Great Northern U. I. 15 and Michelite bean varieties produced top necrosis in many trials. Great Northern U. I. 59 and Great Northern U. I. 123 varieties remained healthy in most of the same trials. In two trials no symptoms appeared on white sweet clover, a reaction characteristic of the pod-distorting strain. Although Grogan and Walker (10) failed to recover the pod-distorting virus from inoculated sweet clover, it was recovered in this investigation in one of the two experiments. In view of these results it is evident that gladiolus is a carrier and one of the overwintering hosts of both strains. While the one case of recovery of the pod-distorting strain from sweet clover is inconclusive, it does indicate that the latter may serve as a masked overwintering carrier.

Characterization of the tobacco ringspot virus from gladiolus.—*Symptoms and host range.*—The gladiolus isolate and the green strain of tobacco ringspot virus were inoculated to 22 species of plants from eight families. The host ranges of the two viruses were identical. The species, in addition to bean, on which symptoms were induced were spinach, cucumber, muskmelon, watermelon, Hubbard squash, crookneck squash, pumpkin, zinnia, corn, pea, soybean, tobacco, *Nicotiana glutinosa* L., and jimsonweed. The symptom syndrome usually included local lesions, top necrosis, sometimes death, and sometimes recovery, with few or no symptoms in new growth. Soybeans were affected with bud blight similar to that described by Allington (2). Symptoms on Hubbard squash consisted only of chlorotic spots on cotyledons, and the virus was not recovered. Chlorotic spots on inoculated leaves were the only symptom on *N. glutinosa*, but both viruses were recovered. No symptoms were induced on gladiolus but each strain was recovered. Cabbage, sweet clover, red clover, crimson clover, and tomato showed no symptoms and the viruses were not recovered. The symptoms on hosts other than bean are given in detail elsewhere (5).

Symptoms induced by both strains differed somewhat on the bean varieties tested. In Idaho Refugee both strains induced local lesions on inoculated leaves, reddening of the pulvinus, systemic necrosis, stunting, unilateral development of trifoliate leaves, mottle, chlorotic rings, top necrosis, and occasionally rapid death (Fig. 2, A). In Great Northern U. I. 59 symptoms included necrotic lesions, top necrosis, reddening of veins, necrosis of petioles, and occasional mottle and unilateral development. Bountiful had many local lesions on inoculated leaves, collapse of primary leaves, and rapid killing of plants. On U. S. No. 5 Refugee, an occasional local lesion on inoculated leaves became elongated when associated with a vein; systemic symptoms were chlorotic and necrotic leaf spots, and stem and petiole necrosis; death of plants was sparse and developed slowly. Stringless Green Refugee had mild symptoms similar to those of U. S. No. 5 Refugee, but more plants were killed slowly.

Corn is a newly recorded host for this virus. Symptoms at 24° to 28° C. consisted of tip necrosis and streaking of the youngest leaf. The yellow strain induced similar but more severe symptoms. In plants inoculated and held at 20°, symptoms were mild mottle and long, narrow, transparent streaks. Necrosis occurred only on inoculated leaves at this temperature. The viruses were recovered readily. Symptoms on bean and corn are illustrated in figure 2.

Properties.—Properties of the gladiolus strain of ringspot virus were studied in comparison with the green strain of tobacco ringspot virus. Expressed juice from plants of *N. tabacum* L. recently infected with the virus concerned was treated and then used to in-

oculate the assay host. Ten black cowpea plants were used as a test unit for the local-lesion assay. The averages of three assays for each property are in table 1. Confirmatory evidence was secured in the thermal-inactivation and tolerance-to-dilution tests by systemic-infection assays with tobacco and Idaho Refugee bean. The thermal inactivation point was usually between 65° and 70°. This agrees fairly closely with the results of Pound (17) with a yellow strain and with those of Henderson and Wingard (11) with several isolates of the virus. When the infectious juice was diluted with distilled water and the plants were inoculated immediately, infection was not obtained at a dilution of 10^{-4} and above, while only a few lesions were obtained at 10^{-3}. Pound (17) gave 10^{-5} and Henderson and Wingard (11) gave 10^{-4} as the dilution end-point. When the expressed infectious juice was stored in stoppered test tubes at 20° C., the virus was inactivated at 6 days in one experiment, at 7 days in another, and a few lesions were secured at 9 days in the third. Pound (17) reported that the yellow ringspot strain with which he worked was inactivated in 5 days. The inactivation points of the green strain were as follows: thermal inactivation, 70° C.; dilution, 10^{-3}; longevity, 7 days in one test and slight activity (0.8 lesion per leaf) at 8 days in another test.

Cross protection tests.—Tobacco plants were inoculated with a given virus strain—the gladiolus, the yellow, or the green strain of tobacco mosaic virus. When signs of recovery appeared, systemically infected leaves were selected for inoculation with another of the respective viruses. Lack of local lesions on inoculated leaves was taken as in indication of cross protection. Although the yellow and green strains protected completely against the gladiolus strain, the latter in one case did not protect against the yellow strain. This type of one-way protection was also observed by Price (20).

Insect transmission.—The green peach aphid, *Myzus persicae* Sulz., was studied as a possible vector of the ringspot strain isolated from gladiolus. Nonviruliferous aphids were raised on healthy tobacco plants and then transferred to an infected tobacco plant. After the aphids had fed for a week, a leaf with 30 to 60 aphids was transferred to a cardboard strip and the strip, but not the infected leaf, was brought into contact with a healthy tobacco plant. As the leaf wilted, the aphids detached themselves and migrated to the healthy plant. The aphids were killed by fumigation after feeding for not less than one week. In three trials no visible transmission to tobacco plants was obtained, nor was any ringspot virus recovered to either tobacco or cowpea. Good recovery was made from the original virus source for the insects.

CHARACTERIZATION OF THE GLADIOLUS STRAIN OF CUCUMBER VIRUS.—*Symptoms and host range.*—Cabbage, pea, bean, white sweet clover, red clover, crimson clover, and soybean produced no symptoms when inoculated with the gladiolus strain of cucumber virus, and the virus could not be recovered from inoculated plants. Symptoms on many of the susceptible hosts were identical when inoculated with either the gladiolus or the typical strain of cucumber virus. Slight differences in symptoms were found in some cases and occasionally one virus was recovered after inoculation and the other was not. Detailed descriptions of symptoms on various hosts are published elsewhere (5).

To make an additional comparison, the spinach varieties Nobel and Virginia Savoy were inoculated and kept at 20° and 28° C. Virginia Savoy had been found in this laboratory by Cheo (8) to be highly resistant at 20° and to show little resistance at 28°. Nobel be-

TABLE 1.—*Thermal-inactivation, tolerance-to-dilution, and longevity-in-vitro tests with the gladiolus strain of tobacco ringspot virus; cowpea as the assay host*

Thermal inactivation		Tolerance to dilution		Longevity *in vitro*	
Temp. ° C.	Lesions per leaf[a]	Dilution	Lesions per leaf[a]	Days	Lesions per leaf[a]
Untr.	236.7	0	123.9	0	115.1
50	271.7	10^{-1}	75.0	1	134.2
55	171.3	10^{-2}	12.3	2	67.5
60	94.2	10^{-3}	0.4	3	37.6
65	0.7	10^{-4}	0.0	4	37.6
70	0.0	10^{-5}	0.0	5	12.1
75	0.0	10^{-6}	0.0	6	6.1
80	0.0	.....	.....	7	8.0
				8	1.0

[a] Each value is the weighted average of results from three experiments.

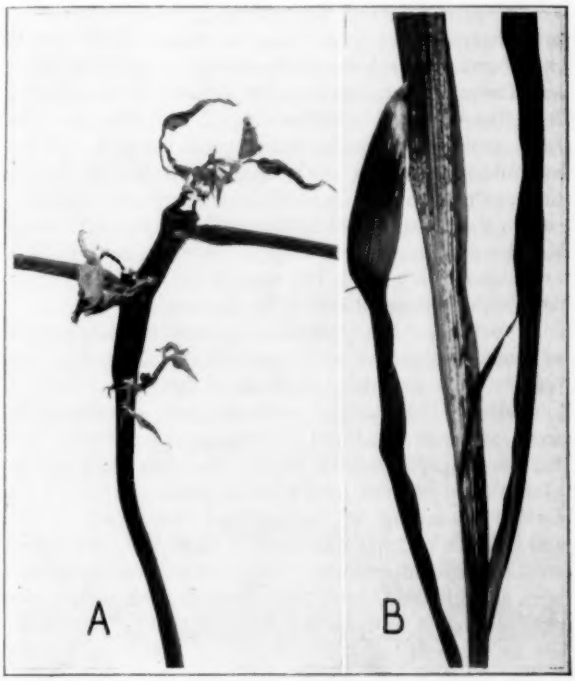

FIG. 2. Symptoms incited on bean and corn by the tobacco ringspot virus. A, stem enlargement and leaf drop following top necrosis on Idaho Refugee bean. B, chlorosis on Golden Cross Bantam corn.

TABLE 2.—*Thermal-inactivation, tolerance-to-dilution, and longevity-in-vitro tests with the gladiolus strain of cucumber virus 1 with cowpea as the assay host*

Thermal inactivation		Tolerance to dilution		Longevity *in vitro*	
Temp. ° C.	Lesions per leaf[a]	Dilution	Lesions per leaf[a]	Days	Lesions per leaf[a]
Untr.	55.7	0	93.8	0	56.0
50	33.2	10^{-1}	76.0	2	4.9
55	2.0	10^{-2}	28.4	3	1.6
60	0.7	10^{-3}	7.1	4	0.6
65	0.3	10^{-4}	1.7	5	0.5
70	0.03	10^{-5}	0.6	6	0.03
75	0.03	10^{-6}	0.1	7	0.0
80	0.0	10^{-7}	0.0	—	—

[a] Each value is the weighted average of results from three experiments.

came badly diseased at both temperatures; the resistant variety Virginia Savoy had no virus symptoms at 20° but was severely affected at 28°.

Members of the Cucurbitaceae and Solanaceae, when inoculated with the cucumber strain from gladiolus, usually produced symptoms typical of cucumber virus 1. In general the legumes were not affected. This is also essentially true for the typical strain of cucumber virus 1. Both isolates gave small, reddish, local lesions on cowpea, typical of those described by Whipple and Walker (23).

Symptoms on hybrid sweet corn of the Golden Cross Bantam variety were small dots or spots on new leaves, streaking, and necrosis of the growing leaf tip, often resulting in death of the plant. In some trials much more necrosis was noted than in others. To investigate this further, mechanical inoculations were made to sweet corn and the plants were kept at 20° and 28° C. Both the typical cucumber virus 1 and the strain isolated from gladiolus gave chlorotic streaks, necrosis, and complete killing at the high temperatures. At 28° the strain that was isolated from gladiolus was more severe than the typical strain; at 20° there was a mild streaking with a great deal of chlorsis, and only an occasional plant died, but symptoms were much more severe than those induced by the typical strain.

Properties.—The properties of the gladiolus strain of cucumber virus were studied to determine how closely they conformed to those of the typical strain of cucumber virus 1. The methods were essentially the same as those employed in making the property tests for the ringspot strains, except that the black cowpea plants were covered for 24 hr. in advance of the inoculations according to the method developed by Sill (21). With at least one trial in each test, the typical strain of cucumber virus 1 was utilized for comparison with the gladiolus isolation. Ten cowpea plants were used as a test unit for local-lesion assay. The results are in table 2.

The thermal inactivation point of the gladiolus strain of cucumber virus was between 60° and 80° C., when exposed for 10 min. The virus was active above 70° C. in only one trial. There is a wide range in the thermal inactivation end points reported by other investigators. For instance, Ainsworth (1) reported 60° and Holmes (12) 70° to 80°. In two dilution experiments no infection was secured at 10^{-6}; in the third, none at 10^{-7}. When the expressed infectious juice was stored in stoppered test tubes at 20° C. the virus was inactivated at 6 days in two experiments and at 7 days in the third. This time is in agreement with that reported for cucumber virus 1 by Whipple and Walker (23) but is slightly longer than that given by Ainsworth (1) and by Holmes (12). In the present investigation the inactivation points for the typical strain of cucumber virus 1 were: thermal inactivation, 70°; tolerance to dilution, 10^{-6}; longevity *in vitro*, 6 days.

Cross protection tests.—The gladiolus strain of cucumber virus produced a typical cucumber-mosaic mottle on zinnia. This allowed the infected zinnia to be tested for protection against Price's strain 6 of cucumber virus 1 (19). Four tests were run by inoculating zinnia plants with the gladiolus isolate used in other property tests. After 2 to 3 weeks, when the plants had developed a systemic mottle, the indicator strain was inoculated to four to six mottled leaves of each zinnia plant. In each trial, a minimum of 10 treated plants was used; 10 plants left as controls were inoculated with the indicator strain at the same time as the last inoculations were made on the mottled plants; and a number of plants were noninoculated. Excellent protection was evident in each trial. Trial 1 developed an average of 13.8 local lesions per control leaf but none on the previously infected leaves. In trials 2, 3, and 4 no lesions developed on the leaves of previously inoculated plants, whereas the controls had 15.3, 14.8, and 24.0 lesions per leaf, respectively. Noninoculated plants remained healthy in all cases.

Only partial protection was found when a second isolate from gladiolus was used. This isolate allowed the indicator strain to incite yellow, chlorotic areas on zinnia in two trials. Cross protection tests were then set up using the strain of cucumber mosaic virus reported by Whipple and Walker (23) as pathogenic on bean and pea. The second isolate from gladiolus was first inoculated into ten young plants of *N. tabacum* L. and the plants were allowed to develop systemic symptoms. Fourteen days later the strain pathogenic to pea and bean was inoculated into the leaves containing the systemic symptoms. After 28 days recovery was attempted from the new leaves of the plant to Idaho Refugee bean plants; 68 bean plants showed no symptoms. One plant was questionable, but no symptoms were obtained when recovery was attempted by inoculation from this plant to tobacco. One later trial on zinnia with the same isolate gave much better cross protection with an average of only four indistinct lesions (less than one per leaf) on four plants, whereas the control plants averaged 24 lesions per leaf.

Insect transmission.—The green peach aphid, *Myzus persicae* Sulz., was studied as a possible vector of the cucumber strain isolated from gladiolus. One trial

contained only tobacco plants but two other trials included three plants each of tomato and tobacco. The transfer was successful to all plants but one tobacco plant. The virus was recovered from tobacco and transmitted mechanically to both cowpea and *N. glutinosa* L. or to the F_1 hybrid between the latter and *N. tabacum* L.

ATTEMPT TO REPRODUCE WHITE BREAK.—A close relationship was found between white break symptoms on gladiolus and the presence of one or more of the viruses characterized in this work. In an attempt to reproduce the flower break, various combinations of the viruses were inoculated to young gladiolus plants when the leaves were 4 to 8 in. long. A minimum of nine healthy gladiolus plants was used in each treatment and 15 plants were left as healthy controls. An occasional plant in the various treatments and in the control had symptoms of white break on foliage and on the blossoms. The percentage of inoculated plants showing white break was not greater than that in the noninoculated group. Brierley and Doolittle (7) reported that cucumber mosaic strains experimentally introduced into tulips produced no recognizable symptoms in the current season but induced flower break in the following year. They also reported this to be true of many of the tulip viruses. In view of these facts the corms from the gladiolus plants in this investigation were kept and grown the following season, but the results were again inconclusive.

DISCUSSION.—Smith and Brierley (22) reported the occurrence of cucumber virus 1 in the genus *Sparaxis* of the Iridaceae. This study and that of Berkeley (3) show that *Gladiolus* is another genus in the family found carrying the virus. In various tests, the strain recovered from gladiolus in Wisconsin resembled the typical strain and was noninfectious on bean.

The strain of tobacco ringspot from gladiolus was also characterized by means of host symptoms, host range, cross protection tests, and properties. The bud blight of soybean was typical of that described by Allington (2). The symptoms on bean were not unlike those described by various authors for tobacco ringspot (11, 13, 16). Blodgett and Schultz (4) described a disease of bean in Idaho with symptoms very similar to those obtained in this investigation when mechanical inoculations were made from gladiolus. The symptoms they described may well have been the result of infection by the tobacco ringspot virus, alone or in combination with a strain or strains of yellow bean mosaic. With the knowledge that gladiolus is a natural reservoir of tobacco ringspot virus, it would not be surprising to find it more often on beans in the field than was formerly supposed. Transmission of the gladiolus isolate to sweet corn is believed to be the first report of this virus occurring naturally in one monocotyledonous plant and capable of infecting another. Other strains of tobacco ringspot virus, inoculated to sweet corn, were infectious in all cases, and the yellow strain used incited a very severe disease with symptoms suggestive of those of some other streak and stripe diseases described on this host. Inspection of corn or sweet corn fields may yield cases of natural infection by the tobacco ringspot virus.

During the first season neither the gladiolus isolate of the ringspot virus nor the gladiolus isolate of cucumber virus 1 gave symptoms on gladiolus when reinoculated to healthy plants. Possibly one or the other of these viruses contributes to the effect of white break in gladiolus, or another undetermined entity may be involved. Additional work on the relation of these viruses to white break would seem to be justified by the high correlation between their presence and the white break symptoms of gladiolus.

SUMMARY

Two viruses previously unreported in gladiolus have been isolated and identified: one as a strain of cucumber virus 1 on the basis of host range, symptoms, properties, transmission, and cross protection; the other as a strain of tobacco ringspot virus, determined in the same manner as that of the gladiolus isolate of cucumber virus 1. This report and that of Berkeley (3) are the first upon the isolation of cucumber virus 1 from gladiolus, although one other genus in the Iridaceae (*Sparaxis*) has been previously reported to carry the virus. It is believed that this is the first report of tobacco ringspot virus occurring naturally in a monocotyledonous plant. Corn was found to be a host of the gladiolus isolate and of the typical strain of cucumber virus 1. It was also a host of the gladiolus isolate and of two other isolates of the tobacco ringspot virus.

A close relationship was found between gladiolus plants which contained the two viruses and those having typical white break symptoms. The exact relation of these viruses to white break was not determined and attempts to reproduce the disease by inoculating them into healthy gladiolus plants were unsuccessful.

Bean virus 2, the incitant of yellow bean mosaic, was also found to occur naturally in gladiolus. Several of the isolates appeared to belong to the pod-distorting strain. Gladiolus is thus one of the overwintering hosts of the typical and of the pod-distorting strain of this virus.

Isolations of the viruses from dormant corms failed although the plants concerned were infected with white break the previous year. Isolations were successful, however, when made from the first young leaves that appeared when the corms germinated.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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LYSIS IN RUST UREDOSPORE GERM TUBES[1]

M. L. Gattani[2]

Lysis among bacteria is a very common phenomenon and may take place as a result of antagonistic or bactericidal action. In fungi, Chudiakov (2) reported lysis of different species of *Fusarium* and other fungi as a result of antagonistic action of two soil bacteria. Lysis of the promycelia of the germinating spores of *Ustilago zeae* has been reported by Chilton (1) and Gattani (4), and in *Sphacelotheca sorghi* by Laskaris (3). In both these fungi, however, the tendency of the promycelia of the chlamydospores to lyse was restricted to certain crosses and was due to a lethal factor or factors in one of the parental lines. So far as the author is aware, no other report is available of lysis among the germinating spores of fungi. The present studies deal with lysis in the uredospores of some rusts, induced as a result of the action of a fungicide. A brief note was published in 1950 (5) and herein are additional observations on the lysis of uredospores of some other rusts.

Material and methods.—Mature viable uredospores of *Puccinia graminis tritici*, *P. triticina*, *P. glumarum*, *P. carthami*, *Melampsora lini*, and *Uromyces decoratus* were used. The fungicide was a dilute suspension of hydrophobic colloidal sulphur, the stock solution of which was prepared as follows.

One lb. powdered quicklime was slaked with ½ gal. water. To the slaking lime 2 lb. sulphur and ½ gal. water were added and the mixture was boiled for about ½ hr. One and one-half lb. molasses were then added and the whole stirred while still hot until the molasses dissolved. Stock dilutions of 10,000, 2,000, 1,000, 200, and 100 p.p.m. were prepared and kept in stoppered bottles. Experimental dilutions of 100, 20, 10, 2, and 1 p.p.m. of the fungicide in 25 ml. water agar were prepared in sterile Petri plates. Control plates contained water without fungicide. Mature spores of the different rusts were dusted on the surface of the hardened agar and spread about with the rounded end of a test tube. Plates containing uredospores of *P. graminis tritici*, *P. triticina*, *P. carthami*, and *Uromyces decoratus* were incubated at 18°C., those of *P. glumarum* at 9° to 10° C., and those of *Melampsora lini* at 12° to 14° C.

Experimental results.—Uredospores of all the rust species germinated normally on water agar without the fungicide. Almost 90 per cent of the spores of the four species incubated at 18° C. germinated within 2 hr. and germ tubes were more than 400 μ long within 18 hr. About 80 per cent of the spores of *P. glumarum* germinated within 2 hr. at 9° C., and their germ tubes were more than 300 μ long within 12 hr. More than 60 per cent of the spores of *M. lini* germinated at 12°–14° C.

When 1 p.p.m. fungicide was present, uredospores of *P. triticina*, *P. carthami*, and *U. decoratus* germi-

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[2]Plant Pathologist, Department of Agriculture, Government of Rajasthan, Bharatpur, India.

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nated normally and no lysis of the germ tubes was noted. Spores of *P. graminis tritici* produced shorter germ tubes than when on the control medium; otherwise their germination was normal. *P. glumarum* was sensitive to 1 p.p.m. of the fungicide and lysis was evident after the germ tubes were approximately 20 μ long. Germ tubes of *M. lini* were longer than 50 μ before lysis occurred.

With increase of concentration of fungicide to 2 p.p.m., lysis of 70–80 per cent of the germ tubes was observed in some species (Fig. 1). Germ tubes of *P. graminis tritici* and *P. triticina* attained an average length of 75 μ within 18 hr., after which lysis occurred in 70 per cent of the tubes. *M. lini* produced germ tubes 25–40 μ long before 80 per cent of them lysed. In two species, *P. carthami* and *U. decoratus*, germination was normal except for short germ tubes in the first species. Uredospores of *P. glumarum*, however, did not germinate when 2 p.p.m. fungicide were in the medium.

Uredospores of some species germinated on an agar with 10 p.p.m. of the fungicide; those of *P. glumarum* and *Melampsora lini* did not. Germ tubes of *U. decoratus* reached an average length of 35μ before lysis was observed. These germ tubes were almost thrice as wide as the normal germ tubes in the control plate. Those of *P. graminis tritici* were approximately 10–15 μ long when lysis began, although occasionally it began as the germ tubes were forming. Germ tubes in this species were almost twice as broad as those in the control plates. In *P. carthami* the germ tubes attained an average length of about 20 μ before lysis began. In *M. lini* a halo of protoplasm around some of the spores seemed to be due to lysis of spore contents before any germ tubes were formed.

Concentrations of 20 and 100 p.p.m. of the fungicide inhibited uredospore germination in all the rust species. To determine whether uredospore viability was destroyed, the spores were removed from the medium, placed in distilled water, and observed for germination. Ten per cent of uredospores of *P. graminis tritici* germinated in distilled water after having been on agar with 20 p.p.m. fungicide for 24 hr. No spores germinated after 48 hr. on such agar. Stem-rust spores that had been on agar with 100 p.p.m. fungicide for 24 hr. failed to germinate when transferred to distilled water.

Lysis sometimes occurred at the side of a germ tube, sometimes at the tips, and sometimes in the first rudiments of germ tubes. When lysis occurred the germ tube stopped growing and the affected part became swollen. Protoplasm accumulated and eventually the tube burst. Thus lysis was lethal to the fungus. Spores with lytic germ tubes were transferred to distilled water, but they failed to produce other healthy and normal germ tubes.

DISCUSSION.—Lysis of the germinating uredospores of the six rusts studied may occur as a result of the action of a fungicide. Rust species differ in their sensitivity to the fungicide, *P. glumarum* and *M. lini* being more sensitive to its action than *P. graminis tritici*, *P. triticina*, *P. carthami*, and *U. decoratus*. Spore germination may begin in low concentrations of the fungicide, but lysis eventually destroys the fungus. Spores with lytic germ tubes when transferred to distilled water failed to recover and did not produce other germ tubes.

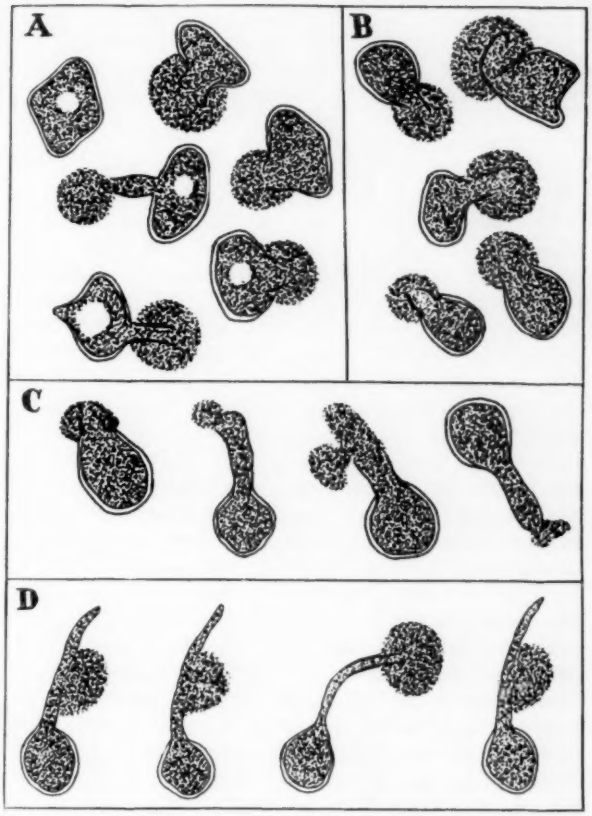

FIG. 1. Lysis in germ tubes of rust spores. A. *Puccinia graminis tritici* (×300). B. *Puccinia triticina* (×300). C. *Uromyces decoratus* (×530). D. *Melampsora lini* (×300).

DEPARTMENT OF AGRICULTURE, GOVERNMENT OF RAJASTHAN
BHARATPUR, INDIA

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CHEMOTHERAPEUTIC INVESTIGATIONS ON THE COMMON BACTERIAL BLIGHT OF BEANS[1]

A. E. Dimond, E. M. Stoddard, and R. A. Chapman

Chemotherapeutants may act in several ways in alleviating disease (10, 11). They may affect the pathogen directly. The nitrosopyrazoles (13), 8-quinolinol and its derivatives (19), $CuSO_4$ (17), and sulfur (18) are all compounds with direct fungitoxicity which have proven to have chemotherapeutic value. They may affect the pathogen indirectly by inactivating the toxins it produces, an approach which has been studied by Howard and his co-workers (7, 8, 12). Alternatively, the compound may act, not on the pathogen but on the host, increasing its resistance to disease by alteration of protoplasmic factors or nutritional balance. The host may also alter the compound to a configuration more toxic to the pathogen.

In view of the many ways in which a chemotherapeutant might act, it seemed advisable to develop first a broad method of assessment in which the over-all effect of a compound on the severity of a disease could be assessed quantitatively. By whatever means a compound acts, its activity can be detected if it be applied to inoculated test plants and distributed in them. Differences between the response of a plant treated with the effective compound and that of the control will thus indicate chemotherapeutic activity, even if little can be said about the means by which the compound produces its effect. The development of such a method was the first objective of this study.

Given the method, the second objective was to assess the activity of several compounds in order to determine what possibilities exist at this time for control of bean blight caused by *Xanthomonas phaseoli*, and, if possible, to obtain clues as to which kinds of compounds are most promising for further study.

MATERIALS AND METHODS.—After a preliminary survey Red Kidney beans, highly and uniformly susceptible to blight, were used throughout the work. Plants were grown in sand in 4-in. pots, from three to five plants to a pot. Because previous experiments (15) had shown that symptoms are more pronounced when nutrient levels are low, plants were maintained at a nutritional level optimal for blight development by feeding them every other day with 0.1 × Hoagland's solution. An amount slightly more than the pot would retain, 50 ml., was used for each feeding.

Goss (9) noted that the higher the temperature, the more rapidly local lesions of common and halo blight appeared. In our experiments, confined to studies on systemic symptoms, elevated temperatures likewise hastened the appearance of wilt symptoms. For this reason the experiments were in a greenhouse thermostatically controlled at 25° C., except during summer months when ambient temperatures exceeded this value. No influence of light or humidity on the rate of appearance of systemic symptoms was noted; hence, no effort was made to control these factors.

Inoculum consisted of 48-hr. cultures of *Xanthomonas phaseoli*, obtained through the courtesy of Dr. W. H. Burkholder. Originally we hoped to inoculate plants so as to produce local lesions and thus obtain a quantitative measure of effectiveness through lesion count. Because the number of local lesions could not be standardized on a routine experimental basis, studies were made on plants inoculated so as to produce a systemic infection. The youngest node on the plant was stabbed with a needle bearing inoculum when the second pair of trifoliate leaves was unfolding.

In studying the methods and timing of application, chemotherapeutants usually were applied to the plant at maximum nonphytotoxic strength, each pot being watered with 50 ml. of solution per day for 10 days.

After suitable incubation, usually for 2 weeks, treatments were assessed in terms of 1) the percentage of plants which wilted because of bacterial blight and 2) the disease index, in which symptom severity in treated plants is expressed relative to that in checks. The latter method involved grading individual leaves on each plant for symptom severity on a scale ranging from zero (no symptoms) to four (complete leaf collapse), establishing the mean grade for the plot and the treatment, and computing the index by dividing the mean grade for a treatment by that for the check plots.

DEVELOPMENT OF A BIOASSAY FOR CHEMOTHERAPY.[2] — *Methods of administering compounds.* — By what method of introducing compounds into plants are the chemotherapeutic properties of compounds made most manifest? The two most likely methods consist of injecting the compound into stems and watering the soil with solutions of it so that the roots may absorb it. In a number of experiments these methods were compared and the results consistently indicated that watering plants with the solutions is the preferred method of application. In a typical pair of experiments, 21 treatments were applied to randomized plots consisting of

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[2] Salicylic acid, which had previously been found an effective therapeutant against bean blight (5, 6), in the course of these investigations became steadily less effective. The cause of this was a steady increase in pathogenicity of the cultures used as inoculum. In the experiments described herein, variations in the performance of salicylic acid always resulted from uncontrolled change in pathogenicity.

TABLE 1.—*The relation of efficacy of treatment to the method of applying chemotherapeutic treatments for common blight of beans*

Compound	Concentration (p.p.m.)	Disease index A Watering	Disease index B Injection
2-(4-morpholinyl)-ethyl phenyl ketone[a]	125	0.29**	0.68
Benzoic acid	500	0.34**	0.81
Sorbic acid[a]	125	0.42**	0.65
Auramine	500	0.47*	0.72
Ethylene urea[b]	63	0.53*	0.56
Benzoyl methyl thiocyanate[a]	63	0.54*	0.60
Butoxypolypropylene glycol[a]	8	0.56	0.66
2-(1-ethylamyl)-2-imidazoline-1-polyglycol[a]	125	0.56*	0.70
8-quinolinol phosphate[c]	500	0.63	0.65
Calcium chloride	1000	0.63	0.85
Tergitol-4-(Na sulfate deriv. of 2-methyl-7-ethyl undecanol-4)[a]	250	0.65	0.73
Potassium permanganate	1000	0.66	0.65
n-Octadecyl trimethyl ammonium bromide[b]	666	0.71	0.54
Crotonic acid[a]	125	0.73	0.69
Protocatechuic acid[b]	500	0.75	0.80
Salicylic acid	250	0.77	0.76
N-(4-nitrophenyl)-3,4-dichloro benzene sulfonamide[b]	15.5	0.78	0.62
Dehydranone[a]	125	0.80	0.56
Cyclopentenyl butyldithiocarbamate[a]	63	0.85	0.73
8-quinolinol salicylate[d]	125	0.96	0.92
Check		1.00	1.00
L.S.D.	$P_{(0.05)} = 0.40$		n. s.
	$P_{(0.01)} = 0.53$		n. s.

[a]Supplied by Carbide and Carbon Chemicals Co., New York City.
[b]Supplied by Rohm & Haas Co., Philadelphia, Pa.
[c]Supplied by Darsyn Laboratories, Paterson, N. J.
[d]Supplied by Mallinckrodt Chemical Works, St. Louis, Mo.

three plants each. Each treatment was applied to five replicate plots. In one experiment of the pair the plants were watered with solutions of the compounds. In the other, the same compounds at the same concentrations were injected into the plant with a hypodermic needle, as much solution as possible (about 0.2 ml.) being forced into the vascular region of the stem without splitting it. All plants were inoculated one day before chemotherapeutic treatment was begun and treatments were applied each day for 10 days. After incubation the disease index was estimated. The summarized results of these two experiments are in table 1.

Obviously it was the purpose of this pair of experiments to compare the value of two methods of applying compounds on a routine basis, not to attempt to apply a compound so that the same amount was taken into the plant. There is no ready means of measuring how much chemotherapeutant is absorbed by the roots of a plant.

Several compounds significantly reduced the severity of bean blight when plants were watered with solutions of them. This effect is indicated not only by the analysis of variance (value of F for treatments was 1.813 where $F_{(0.05)}$ was 1.70), but also by estimating the "just significant difference" (Table 1). In contrast, when compounds were injected into stems, no treatment caused significant reduction in severity of disease (value of F for treatments was 0.733 where $F_{(0.05)}$ was 1.70). This resulted from the fact that effects were not consistent when compounds were injected into stems. In these experiments it was evidently preferable to water plants with solutions of the test compounds rather than to inject these materials into plant stems.

TABLE 2.—*Relation of timing of treatment and number of applications to the efficacy of salicylic acid as a chemotherapeutant for common blight of beans*

Time and number of applications (successive days)	Percentage of plants wilted	Disease index
Before inoculation		
8	95	0.63
4	91	0.77
2	100	1.08
1	94	0.65
Check		
0	100	1.00
After inoculation		
1	95	0.82
2	83	0.45
4	92	0.47
8	30	0.48

Timing of treatment in relation to inoculation.—When ought chemotherapeutic treatment to be begun in relation to time of inoculation, in order to obtain the greatest differentiation of compounds? To answer this, experiments were set up in which the timing and the number of applications were varied in relation to the time of inoculation, using salicylic acid. All treatments were made by watering plants with solutions. Each treatment was applied to a minimum of 33 plants. Plants were all inoculated from one batch of inoculum and on the same day—the day following last application of salicylic acid or that preceding the first application (Table 2). Severity of disease was estimated and disease indexes were computed for plant, plot, and treatment. Results are in table 2.

When plants were treated before they were inoculated, there was but little effect. In contrast, the disease picture altered in marked fashion in plants treated after inoculation as the number of applications was increased. Thus severe wilt symptoms appeared on most of the plants receiving only one application of salicylic acid; mild symptoms appeared on plants having two or four applications. Only a few plants wilted after eight applications, but symptoms were severe on those few.

These results suggest strongly that a compound will be rated more favorably when applied after inoculation of the plant, and, moreover, that the number of applications of a compound should be more than a certain critical minimum. As judged by the percentage of plants having wilting symptoms, several applications should be made on successive days if maximum dif-

TABLE 3.—*The presence of bacteria in plants treated with effective chemotherapeutants as determined by their recovery in culture*

Treatment	Symptomless plants		
	Percentage	Number of isolations attempted	Percentage of positive isolations
Lauryl isoquinolinium bromide	69	23	65
Zinc sulfate	89	9	56
Calcium chloride	70	11	55
Salicylic acid	86	25	48
Auramine	37	14	21
Check	5	5	100

TABLE 4.—*The relation between pathogenicity of cultures of Xanthomonas phaseoli and the efficacy of salicylic acid as a chemotherapeutant*

Experiment number and date	Number of days after inoculation for first symptoms	Disease index of plants treated with salicylic acid (percentage of check)
3—November 18, 1947	7	0.06
32—April 8, 1948	8	0.11
42—July 28, 1948	5	0.23
46—August 24, 1948	3	0.77
56—December 10, 1948	3	0.82
63—January 18, 1949	3	0.85

terences between an effective treatment and the check are to be obtained.

Condition of bacteria in inoculated plants which do not wilt under treatment with a chemotherapeutant.—When plants are treated with effective compounds, either they do not wilt or wilting is slight. One may inquire whether bacteria are multiplying in the treated plants but are not pathogenic, or whether treatment has limited multiplication of bacteria in the host. Accordingly, plants were watered with solutions of the compound after inoculation, and attempts were made to isolate bacteria from the plants after wilting symptoms had developed in check plants and in ineffective treatments. In all cases attempts at cultures were made from plants with no wilt symptoms, except check plants. Results are in table 3.

Apparently the first four compounds in table 3 do not act by killing bacteria in the plant, since bacteria are frequently isolated from symptomless plants. These compounds probably are active because they reduce the pathogenicity of bacteria without killing them. In contrast, bacteria could seldom be isolated from plants which showed no wilt after treatment with auramine. Probably this compound is bactericidal in the host.

Effect of variations in pathogenicity of Xanthomonas phaseoli upon the efficacy of chemotherapeutic treatment.—Variations in pathogenicity of cultures may arise, in part, by genetic mechanisms; other factors may be age of a culture, the frequency with which it is transferred, or the time since it was last put through a living host (2).

When received from Dr. Burkholder, the culture of *Xanthomonas phaseoli* was immediately transferred, but no other transfers were made for some time. When, finally, active studies on bacterial blight of bean were begun, the stock culture was transferred more regularly, and as the work progressed and more experiments were run, this was done every 48 or 72 hr.

In a review of the results obtained over 14 months, two relations stand out: 1) the pathogenicity of the culture gradually increased during the first 8 months of the investigation and remained constant thereafter, so far as could be determined; and 2) the efficacy of salicylic acid as a chemotherapeutic treatment steadily decreased. The data on which these statements are based are in table 4. Evidently thought must be given to the influence which variations in pathogenicity will have upon the results of experiments dealing with chemotherapy. The pathogenicity of the test organisms should be regularly assayed, and the procedure for producing inoculum should be well standardized so as to minimize variations from this cause.

AN EVALUATION OF COMPOUNDS AS CHEMOTHERAPEUTANTS FOR BEAN BLIGHT.—In a series of experiments, a number of compounds were bioassayed as chemotherapeutants for bean blight. Those whose activity is reported were all assessed by application of solutions to plants each day for ten successive days following inoculation. Because the pathogenicity of cultures varied during the experiments, the value of compounds given a high rating may have been overestimated. However, those given low ratings may be rejected. Compounds were selected initially on the basis of their solubility in water and known activity as fungicides or bactericides. Subsequent selections were based in part upon compounds previously found to have chemotherapeutic promise against other diseases (5, 6, 14, 19), and in part upon random selections to determine whether other useful criteria for selecting compounds could be discovered.

Because salicylic acid was one of the first compounds found to exert a pronounced chemotherapeutic action against bean blight (5, 6), the chemotherapeutic potency of a number of salicylates was examined. In some experiments 8-quinolinol salicylate produced plants having indices half those of check plants. Sodium salicylate reduced slightly the extent of attack by the common blight organism. Acetyl salicylic acid, salicylanilide, and phenyl and methyl salicylates were ineffective.

Apart from 8-quinolinol salicylate, mentioned above, the following derivatives of 8-quinolinol were of little value in reducing the severity of bacterial blight of beans: 2-quinolinol, 8-quinolinol, 8-quinolinol-5-sulfonic acid, and the phosphate, benzoate, and sulfate of 8-quinolinol.

Phenols, with the possible exception of *p*-nitrophenol (index 0.56 of control) did not reduce the level of disease significantly. Phenol, hydroquinone, 3,5-dinitro-*o*-cresol, *o*-nitrophenol, protocatechuic acid, catechol, picric acid, and dichloro dihydroxy diphenylmethane were of no value.

Mercury compounds—mercuric chloride, pyridyl mercuric acetate, and phenyl mercury triethanol ammonium lactate—were generally ineffective.

Of the four dyes, auramine, chloramine T, malachite green, and methylene blue, only auramine had a pronounced effect.

None of the sulfonamides used was outstanding. These were sulfanilamide, sulfasuxidine, and N-(4-nitrophenyl)-3,4-dichlorobenzene sulfonamide.

Benzoic acid was significantly effective in certain trials but *p*-aminobenzoic acid was not.

Quaternary ammonium compounds, although said to be efficient bactericides, were not consistently of value. Thus while lauryl isoquinolinium bromide was effective, octadecyl trimethyl ammonium bromide and Roccal were not.

Cyclopentenyl butyl dithiocarbamate and disodium ethylene bisdithiocarbamate were of little value.

A number of miscellaneous compounds were used with varying results. In some experiments both zinc sulfate and calcium chloride significantly reduced the severity of disease. Ethylene urea, sorbic acid, benzoyl methyl thiocyanate, 2-(4-morpholinyl) phenyl ethyl ketone, and 2-(1-ethylamyl)-2-imidazoline polyolycol slightly reduced severity of disease. Ineffective were potassium permanganate, sodium selenate, acetone, thanol, maltose, chloranil, thiourea, isoamyl formate, and dl-mandelic acid.

None of these compounds had sufficient potency as a chemotherapeutant to promise practical control of bacterial blight of beans when virulent strains of the pathogen were involved. It remains to be seen whether such compounds will be found in the future.

Discussion.—The study on bean blight is probably a clear-cut case of chemotherapy of plant diseases. The chemotherapeutant is applied to the roots and inoculation is made just below the growing point of the stem. Thus, when treatment significantly reduces the severity of disease, there can be little doubt that the compound has been absorbed by the root and that this compound or a product derived from it has been translocated to the area of infection. When inoculation precedes treatment, reduction in severity of disease must involve therapy.

These studies have shown that, when plants are watered with a solution of the compound, the chemical behaves more uniformly and in more pronounced fashion as a chemotherapeutant than when the compound is injected into the stem of the plant. The same situation prevails when 8-quinolinol benzoate is used as a chemotherapeutant for Dutch elm disease (4) and also in the chemotherapy of Fusarium wilt of tomatoes.[3] All of the available evidence indicates that injection of compounds into the stem is inefficient for the objectives of chemotherapy.

Whether a chemotherapeutant is more effective if applied before or after inoculation of the plant is of real importance in designing methods for measuring chemotherapeutic potency. It is also of importance in practice if one can say that when bean blight is inoculated so as to produce a systemic infection, effects are more pronounced when inoculation occurs prior to treatment (Table 2). Stoddard, working with the X-disease of peach, has reported similar results (14). X-disease is transmitted experimentally by budding diseased bark or buds into healthy stock. It is quite possible that little translocation of inoculum or of therapeutants can occur across the wound barrier until the tissues have begun to grow together. When the plant is inoculated with the bean blight organism by stabbing a needle through the stem, a wound barrier is also formed. How can it be that in two diseases of this sort, the chemotherapeutant is most effective when applied after inoculation has occurred? For one thing, to eliminate the pathogen at once the therapeutant must be free to move across the wound barrier from surrounding tissues—a process that will proceed slowly until the wound is healed. For another, the therapeutants under consideration here have a short effective life in the plant (Table 2). Because this process goes on, what is in the plant before inoculation may be rendered inactive before it can be translocated across the barrier created by a wound.

When no barrier of crushed or grafted tissue is involved the picture appears quite different. Studies on Dutch elm disease (4) and Fusarium wilt of tomato (3) and carnation (16) have shown clearly that inoculation following treatment is not merely to be preferred; it is a necessity for successful chemotherapy. This relation governs timing of applications in practice if time of inoculation is known.

Considered from the point of view of a compound acting upon a growing mass of inoculum, the study of chemotherapy presents some interesting problems. A unit quantity of chemotherapeutant will be required to inactivate a unit quantity of inoculum. However, bacteria begin to multiply in the host within a few hours of their entry into it (1). If treatment is to be effective the amount of chemotherapeutant which arrives in a given time at the locus of the inoculum (the site of potential infection) must at least be equal to the minimum dose required to prevent the increase in inoculum in this same time. If this does not happen, then the inoculum will increase, however slowly, and infection will spread.

In the present studies continued application of suitable compounds has reduced both the number of affected plants showing wilt symptoms and the severity of the wilt symptoms (Table 2). This indicates a suppression of bacterial invasion, a conclusion which has been confirmed by attempts to culture bacteria from treated plants which fail to wilt (Table 3).

When the pathogenicity of cultures used in these studies increased, the effectiveness of treatment decreased. Salicylic acid was highly effective when moderately pathogenic cultures were employed (Table 4), but quite ineffective when highly pathogenic cultures were used. Bearing this in mind, one may now consider the results in table 2: evidence which indi-

[3] Unpublished work by A. E. Dimond, R. A. Chapman, and E. M. Stoddard.

cates that more than four applications on successive days after inoculation will be required for maximum effectiveness of treatment.

If the inoculum is so pathogenic as to produce symptoms in the plant in less time than that required for absorption of a chemotherapeutic dose, then higher concentrations of chemotherapeutant must be administered. If maximum nonphytotoxic concentrations have already been employed, then effective treatment becomes impossible. We may conclude, therefore, that the relation of the time required for a plant to absorb a chemotherapeutic dose to the incubation period of the pathogen is critical; this relation will spell the success or failure of treatment for one disease even if the compound has previously been found promising as a chemotherapeutant for other diseases.

These studies have evaluated a number of compounds as chemotherapeutants for bean blight. All of the compounds which had value chemotherapeutically have either bactericidal or fungicidal potency, but not all of the compounds having toxicity for microorganisms were of value chemotherapeutically. This result would be expected. Some may not be absorbed by the plant; others may not be translocated. Still others may be detoxified in the plant, and, if their products arrive at the point of infection, these are ineffective. It is probably significant, however, that no compound which does not have toxicity for microorganisms was promising as a chemotherapeutant in these experiments.

SUMMARY AND CONCLUSIONS

The results of a chemotherapeutic investigation on bacterial blight of beans, caused by *Xanthomonas phaseoli*, are presented. A method was developed whereby one may measure by bioassay the potency of a compound as a chemotherapeutant for this disease. Inoculum is stabbed into the youngest node of young bean plants. The plants are then watered with solutions of the test compound at maximum nonphytotoxic strength. After 2 to 3 weeks' incubation, results are obtained by grading individual leaves on plants, computing the mean grade per plot, and relating the resulting grade to treatment.

Compounds were more effective as chemotherapeutants when solutions of them were applied to plants and absorbed through the roots than when the compound was injected with a hypodermic needle into the stem. Similarly, treatments were more effective when applied just after inoculation of the plant than just before, and when applied for several successive days rather than merely for 1 or 2 days.

Attempts to isolate bacteria from treated but nonwilted plants showed that the multiplication of the pathogen had been reduced by treatment and also that pathogenicity of the bacteria had been reduced. An increase in pathogenicity in the inoculum employed in experiments over the period of investigation was of such magnitude that treatments which were originally effective were quite ineffective when used in later experiments. It is suggested that if the pathogenicity of an organism is such that infection develops actively in a time less than is required to build up a chemotherapeutic dose in the plant, a treatment will be ineffective unless the time to establish this dose can be reduced.

A number of compounds were evaluated as chemotherapeutants for bean blight. All of those of value have known bactericidal or fungicidal potency; but many compounds having such potency were not of value as chemotherapeutants.

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

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THE OLIVE TREE AS A HOST OF THE CITRUS-ROOT NEMATODE [1]

R. C. Baines and Gerald Thorne [2]

The citrus-root nematode, *Tylenchulus semipenetrans* Cobb,[3] was first observed on orange roots in California by Hodges in 1912.[4] This nematode has since been found on the roots of 12 species of *Citrus*, as well as on close relatives of *Citrus* such as *Atalantia citroides* Pierre ex Guill., *Fortunella* sp., *Microcitrus* spp., and *Poncirus trifoliata* (Linn.) Raf.[4,5] All of these hosts belong to the family Rutaceae.

Olive roots collected by M. M. Winslow near Hemet, California, in October, 1942, were found by the junior author to be infested with a nematode morphologically identical with *Tylenchulus semipenetrans*. A second lot of olive roots collected near Lindsay, California, by E. C. Moore and received in May, 1944, was also infested with nematode. Allen[6] briefly mentions the occurrence of the citrus nematode on olive in California.

The olive belongs to the family Oleaceae, which is considerably removed botanically from the family Rutaceae. Since a detailed study of the nematode on olive had not been made, it seemed important to determine whether this nematode was pathologically, as well as morphologically, similar to that on citrus.

Inoculation studies.—Nematode larvae for inoculations were obtained from the roots of infested sour orange and Mission olive trees growing in separate fields at Riverside, California. The infested roots were washed quickly in water and then covered with water in a pan for a few hours. The suspensions of larvae thus obtained were placed on filter paper in funnels, and the larvae, after passing through the paper, were collected in vials attached to the base of each funnel. Larvae thus obtained were relatively free from extraneous material.

Experiment 1.—One rooted olive cutting of the Sevillano variety and one small sour-orange seedling of the Standard variety were planted on opposite sides of each of nine small root-observation boxes (Fig. 1).

Fig. 1. Small olive cuttings and orange seedlings in root-observation boxes used for nematode inoculations. At right, one side of wooden box has been removed to expose roots growing against glass side of box. (One-third actual size.)

One week after the trees were planted in the boxes, 260 larvae from olive roots were placed on the roots of each olive and orange tree in three of the boxes. Each tree in three other boxes was inoculated with 5,000 larvae obtained from orange roots. Trees in three other boxes were maintained for controls. All the trees were kept in a greenhouse.

Four months after inoculation, the roots against the glass sides of the boxes were examined for females of *Tylenchulus semipenetrans*. A few females were found on the roots of three orange trees and one olive tree which had been inoculated with larvae from olive roots. Numerous females were found on the three orange trees inoculated with larvae from orange roots, but no infestation was observed on the olive roots in the same boxes. Many of the olive roots appeared to have died, and this may have affected the results.

Nine months after inoculation the roots of the trees were carefully removed and examined for colonies of *Tylenchulus semipenetrans*. Mature females developed on the roots of all the orange and olive trees which

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Paper No. 701, University of California Citrus Experiment Station, Riverside, California.

[2] Associate Plant Pathologist, California Agricultural Experiment Station, and Senior Nematologist, Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, respectively.

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TABLE 1.—*Relative infestation of roots of Sevillano olive and Standard sour orange grown together in observation boxes and inoculated with larvae of Tylenchulus semipenetrans from olive and orange roots.*

Source of inoculum	Observation box No.	Degree of infestation[a]	
		Orange roots	Olive roots
Mission olive roots[b]	1	+++	+
	2	+++	++
	3	++	+
Sour-orange roots[c]	1	+++	+
	2	+++	+
	3	+++	+
Noninoculated (control)	1	0	0
	2	0	0
	3	0	0

[a] O = no females of *T. semipenetrans* on the roots; + = fewer than 1 female per cm. of roots; ++ = 1 to 3 females per cm.; +++ = more than 3 females per cm.
[b] Approximately 260 larvae were used for inoculation of each plant.
[c] Approximately 5,000 larvae were used for inoculation of each plant.

had been inoculated with larvae from olive or orange roots (Table 1). A greater number developed on the orange than on the olive, regardless of the source of the inoculum. However, apparently a higher percentage of larvae from olive infested the two hosts than larvae from orange roots. No infection developed on the control trees.

Experiment 2.—For a study of the effect of host association on infestation with the citrus-root nematode, a Mission olive seedling and a Homosassa sweet-orange seedling were planted together in 6-in. pots. Olive and orange seedlings were also planted separately in other pots. Inoculum was introduced through a glass tube inserted to a depth of about 3 in. in the center of each pot. Two pots of each of the three lots were inoculated 9 and 30 days after planting, each pot receiving approximately 26,300 larvae obtained from sour-orange roots only. A like number of trees similarly potted were maintained for use as noninoculated controls. All the trees were kept in a greenhouse for 5 months and the degree of infestation on the roots was then determined.

The roots of each plant were carefully removed and cut into small pieces. One-gram lots of the roots were then enclosed in cheesecloth bags and suspended in water for 48 hr. in funnels, each of which had a vial attached to its stem (modification of the Baermann method). The number of nematode larvae obtained from 1 gm. of roots and the relative numbers of females found on the roots (Table 2) were less on olive roots than on orange roots, whether the two hosts were growing in the same pot or in separate pots. Larger numbers of larvae were obtained from the olive roots when grown in association with the orange than when grown separately in the pots. The writers believe that such differences should be attributed largely to an increase of the nematodes on orange roots and to subsequent infestation of olive. No infestation developed on the roots of the control trees.

TABLE 2.—*Relative infestation of roots of Mission olive and Homosassa sweet orange when grown together and separately in pots and inoculated with larvae of Tylenchulus semipenetrans from sour-orange roots*

Trees in pots[a]	Degree of infestation with females[b]		Average number of larvae per gm. of roots	
	Olive	Orange	Olive	Orange
Olive and orange	+	++	119	775
Olive	+	—	12	—
Orange	—	++	—	727
Controls (same as above)	0	0	0	0

[a] Each treatment was made in duplicate.
[b] O = no females of *T. semipenetrans* on roots; + = fewer than 1 female per cm. of roots; ++ = 1 to 3 females per cm.

PATHOGENICITY AND MORPHOLOGY.—In the tests reported above the nematodes from olive and orange roots appeared to be similar in pathogenicity, although the larvae from olive were more infective in experiment 1 (Tables 1 and 2). The light infestation obtained on olive roots suggests that the nematode does not infest and reproduce as readily on these roots as on orange roots. Olive roots infested naturally in the field at Riverside, California, also have shown considerably lighter infestation than orange roots infested under similar conditions.

Microscopic examination of specimens of nematodes occurring naturally on olive and orange roots revealed that both were typical of *Tylenchulus semipenetrans* Cobb.[3]

SUMMARY AND CONCLUSIONS

Reciprocal inoculations were made on olive and orange roots with larvae of a similar nematode obtained from these two hosts. Specimens of the nematodes from both olive and orange roots also were examined critically for identification of species. The nematode occurring on olive roots in California apparently is similar morphologically and pathologically to the citrus-root nematode, *Tylenchulus semipenetrans* Cobb, obtained from orange roots.

The citrus-root nematode readily completed its life cycle on olive roots, whether the source of the inoculum was olive or orange. The olive, however, did not appear to be so favorable a host as the orange. The nematode exhibited no apparent host specificity between olive and orange.

The olive tree should be recognized as a host in any program planned for control of the citrus-root nematode.

DIVISION OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION
RIVERSIDE, CALIFORNIA, AND
U. S. NEMATOLOGY FIELD LABORATORY
SALT LAKE CITY, UTAH

ELECTRON-MICROSCOPE STUDIES OF A NEW ORCHID VIRUS COMPLEX[1]

Nelson Newton and David W. Rosberg[2]

Several viruses of *Cattleya* orchids have been reported. Jensen reported a virus causing flower distortion and leaf mottle and malformation. Newton and Rosberg (4) reported two morphologically different particles associated with a virus disease of Cattleya, and Gold (1) reported rod-shaped particles, variable in length, associated with broken flowers of Cattleya.

The present studies are concerned with further electron microscopy and associated investigations of what is believed to be an orchid virus complex infecting *Cattleya* spp.

Several years ago the orchids grown for commercial purposes in the vicinity of Columbus, Ohio, were disfigured by dark-colored areas or streaks in the petals and sepals, some distortion and mottling of blossom petals and lips, and leaf mottle symptoms. A virus was suspected as the causal agent of the disease and extensive pathological and host-range studies were made. Attempts to transmit the disease from orchid to orchid by various means met with apparently only very limited success. Whenever transmission appeared to have been accomplished, symptoms appeared on the plants only after about 12 weeks or longer (5). In an effort to identify the causal agent of this orchid disease, the following studies were made.

MATERIALS AND METHODS.—*Preparation of extracts.*—Leaves of diseased orchid plants were harvested and ground to a fine pulp; this was pressed through four layers of cheesecloth and the raw juice was concentrated and purified by alternate centrifugations at 6,460 g. (low speed) for 30 min., and 19,942 g. (high speed) for 1½ to 2 hr. The process was then repeated. An almost colorless opaque substance, found in the bottoms of the centrifuge tubes following the final high-speed centrifugation, was suspended in 20 cc. of a 0.05-M KH_2PO_4-K_2HPO_4 buffer at pH 7.

For comparison with the diseased extract, juice from the leaves of eight different, apparently healthy orchid plants was extracted separately and purified using differential centrifugation. Of these eight plants, only four yielded the opaque substance.

Electron-microscope studies.—Microdrops of the highly concentrated and purified suspensions from apparently healthy and diseased plants were diluted with distilled water and mounted on electron-microscope viewing screens, which had been coated with a 0.2 per cent solution of Formvar dissolved in ethylene dichloride. The specimens were then platinum-shadowed at an angle of approximately 12° and viewed with a Universal type RCA electron miscroscope.

Serological studies.—Because only comparatively small amounts of purified extracts from abnormal plants were on hand, it was necessary to dilute the concentrated purified materials so that two rabbits could be given five inoculations of 5 cc. each, 3 to 4 days apart. At the end of this schedule, 10 cc. of blood were taken from each animal by heart puncture and the same amount from one noninoculated control animal. All blood samples were allowed to clot for 1 or 2 hr. at room temperature and centrifuged twice to secure the serum, which was then stored in the refrigerator.

The precipitin ring test was used. The homologous antigen was evaluated quantitatively during serological testing. Use of a complete set of controls obviated the necessity of using the serum-absorption method, in which homologous antigen is removed from all sera to be used in the tests.

The serum was first introduced into the usual small-bore precipitin tube and the antigen, prepared in dilutions of 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30, was carefully layered, with a micropipet, over the surface of the serum.

Examination for optical properties.—Purified preparations from both diseased and normal orchids were examined with the polarizing microscope to determine whether or not the material showed streaming birefringence.

RESULTS.—Platinum-shadowed preparations of the suspension revealed that the entities present were definitely rod-shaped structures, many of them very long and sinuous (Fig. 1). In concentrated materials (Fig. 2) these rods existed for the most part in parallel aggregates or in large, nonoriented masses resembling a brush heap. In diluted preparations it was possible to find many isolated particles. Numerous mounts were made of the extracts from diseased plants, and in every case rodlike bodies were found.

Electron-microscope studies of purified extracts of two apparently healthy plants revealed the same types of rodlike bodies. Because rodlike material similar to that shown in figures 1 and 2 has not been reported as a constituent of the extracts of healthy plants, further preparations were made from six very young orchid plants to determine whether or not rod-shaped bodies are present in all apparently healthy plants. Samples from each of these plants were platinum-shadowed and examined with the electron-microscope. Rod-shaped bodies were noted in one of these; one

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[2]Respectively, Battelle Memorial Institute, Columbus, Ohio, and Department of Plant Physiology and Pathology, Texas Agricultural and Mechanical College, College Station, Texas.

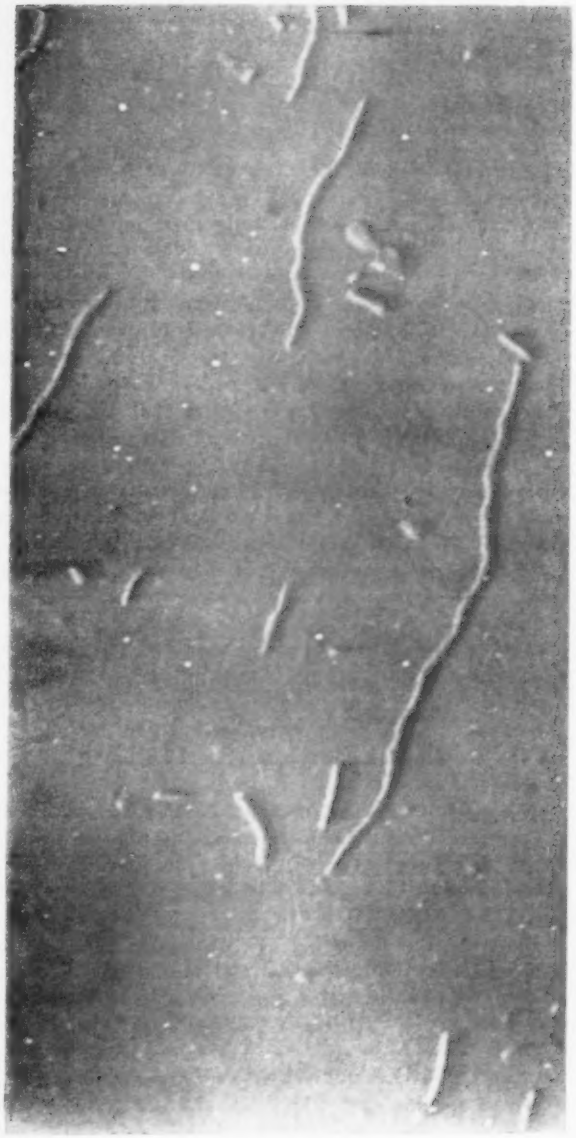

FIG. 1. Virus particles, principally sinuous rods, from diseased Cattleya orchid. The long, threadlike particle (right center) is approximately 1525 mμ long. Platinum-shadowed at approximately 12° angle. (× 46,800)

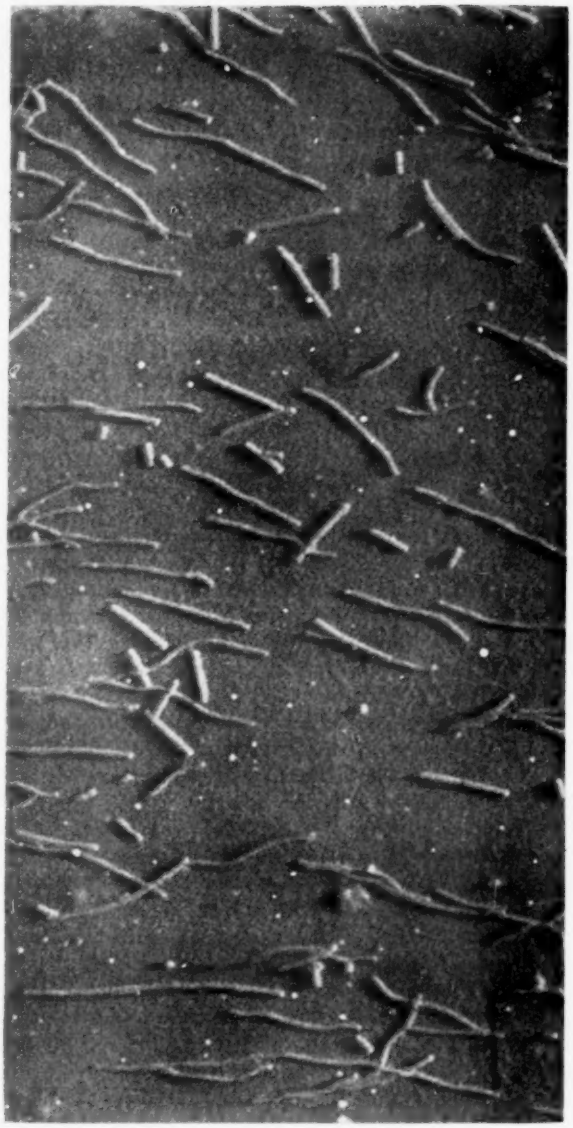

FIG. 2. Sinuous and straight rodlike virus particles from diseased Cattleya orchid. Platinum-shadowed at approximately 12° angle. (× 46,800)

was questionable, having only an occasional isolated rod; and the remaining four showed only occasional traces of plant debris, without rods.

A study of 25 electron micrographs revealed the possibility that two different types of rodlike bodies were present: a straight rod and a longer, sinuous particle.

To determine the basic length or lengths of the entities present, 203 particle-length measurements were made.

The rigid, rodlike forms were approximately 300 mμ long (Fig. 3) and averaged about 24 mμ in diameter. The less rigid, sinuous rods averaged approximately 390 × 21 mμ. Some particles were longer or shorter than the predominating units.

When examined with the polarizing microscope, concentrated and dilute suspensions from diseased orchids showed marked degrees of streaming birefringence, but similar preparations from healthy plants lacked this property.

Exposure of concentrated extracts from diseased plants to an intense beam of light produced a pronounced Tyndall cone, not seen in the normal plant extracts.

Precipitin ring tests (Table 1) reveal a relatively pronounced ring test at a dilution of 1:5 of the anti-

TABLE 1.—*Results of precipitin ring tests with extracts from diseased and healthy orchid plants*

Plant extract	Serum	Antigen dilutions					
		1:5	1:10	1:15	1:20	1:25	1:30
Healthy	Anti-healthy plant	±	±	−−	−−	−−	−−
Healthy	Anti-virus-diseased plant	+	+	±	−−	−−	−−
Virus-diseased	Anti-healthy plant	±	−−	−−	−−	−−	−−
Virus-diseased	Anti-virus-diseased plant	++++	+++	++	+	±	−−
Healthy	Normal	−−	−−	−−	−−	−−	−−
Virus-diseased	Normal	−−	−−	−−	−−	−−	−−

gens, i.e., the orchid virus complex. The reaction becomes progressively less until, at a dilution of 1:25 and 1:30, only a trace of ± reaction is indicated.

Since the antisera were dilute suspensions of the antigen, the extent of dilution of the antigen capable of giving positive results is rather limited. Moreover, plant viruses are rather poor antibody stimulators, in comparison with the animal and human viruses. The results of the ring tests, therefore, support the contention that the diseased material contained an antigen not present in healthy extracts. Homologous antibody was also present, but in no case was a ring test for homologous antibody visible at a dilution greater than 1:10.

DISCUSSION AND CONCLUSIONS.—The studies presented here indicate strongly that the infective agent is a plant-virus complex unreported heretofore. The complex may consist of two different viruses, or two different forms of the same virus. In the light of our present knowledge of the plant viruses, the first hypothesis seems to have more justification. The latter conclusion would imply that this plant virus may have two morphologically different stages in its life cycle. Such complexity in a plant virus has never been demonstrated.

The entities found in extracts from diseased plants are rigid, rodlike forms, approximately 300 × 24 mμ, and sinuous, flexible, rod-shaped forms, approximately 390 × 21 mμ. Forms shorter than 300 mμ are probably produced by the purification techniques employed. The greatest number of short particles are classed as rigid-appearing, rodlike forms (Fig. 3). In gathering data for both graphs, only the particle lengths were considered. Without doubt a number of the small fragments recorded in figure 3 were derived from the longer, sinuous forms. With the fragmentation of sinuous forms the particles arising therefrom appear as straight fractions. Some end-to-end aggregation of the sinuous rods is indicated in figure 4 by the predominance of particles 800–820 mμ long, which is approximately twice the length of the single units.

From a purely pathological standpoint, these studies

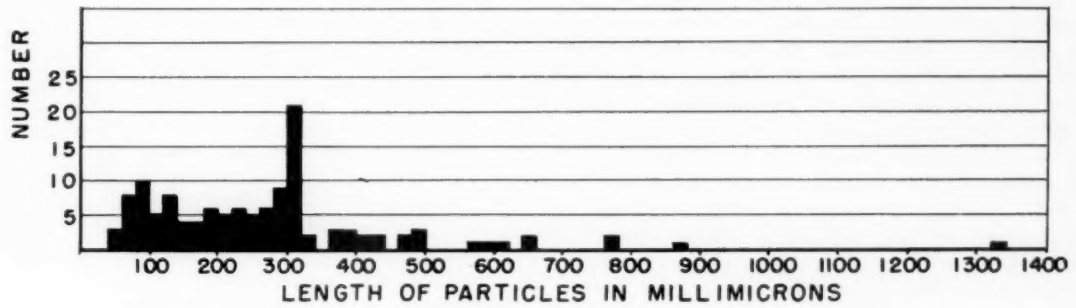

FIG. 3. Length distribution of straight, rigid-appearing rod-shaped particles of virus.

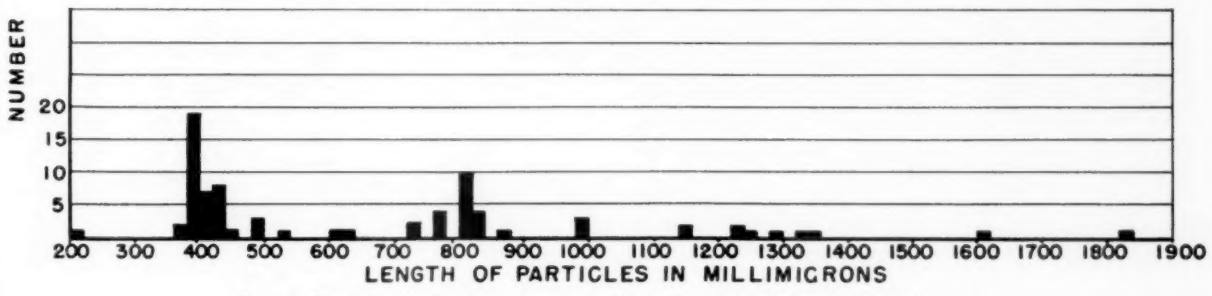

FIG. 4. Length distribution of curved, sinuous rod-shaped particles of virus.

indicate that symptoms of virus infection do not always appear in diseased orchid plants. On the basis of the results presented here, it is assumed that the isolation of rodlike forms from orchid plants indicates virus infection although symptoms may be absent.

Under the polarizing microscope, extracts from diseased plants showed pronounced streaming birefringence. This property is found in colloidal suspensions in which the micelles are particles having one axis longer than the other two. Streaming birefringence was not seen in preparations from plants shown to be healthy by electron-microscope examinations.

Morphologically the shorter, rigid form resembles tobacco mosaic virus, being somewhat larger in over-all dimensions, however. All attempts to transmit the virus to tobacco were negative.

Some morphological similarities are noted between the shorter, rigid-appearing rods found in these studies and those observed by Jensen and Gold (3). The widths of each are the same but the lengths differ by about 20 mμ.

Some interesting speculations regarding the differences in the physiochemical structure arise from a comparison of the dimensions and morphological appearance of forms involved in the orchid virus complex and tobacco mosaic virus. Considering the dimensions of each in relation to the length, it is evident that the shorter viruses, tobacco mosaic virus and the shorter rod forms of the orchid virus complex, are rigid structures. The longer forms found in the orchid virus complex appear to be tenuous and nonrigid. This fact might suggest that flexibility of virus rods is a direct function of their lengths and, to a lesser degree, their diameters. This assumption does not hold, however, when we consider the fact that tobacco mosaic virus aggregates end-to-end but maintains the same rigidity as do the units 280 mμ long. Even with extreme end-to-end aggregation, the rigidity of structure is unaffected. This fact suggests that the length of a virus is unrelated to irregularity of form. It seems much more probable that the degree of rigidity is a function of the physiochemical structure. Possibly the bonding forces holding rod-shaped viruses together as units are qualitatively weaker in some than in others; or the bonds may be of the same strength, but in sinuous forms spaced in such a fashion as to produce a looser, less dense over-all protein structure.

From the initiation of the work until the first platinum-shadowed rodlike forms were observed in the electron microscope, only 12 hr. elapsed. The electron microscope, therefore, can be an invaluable aid in the rapid detection of a plant virosis, as well as being an excellent tool for many other types of virus investigations.

SUMMARY

Electron-microscope studies of centrifugally purified material from abnormal orchid plants revealed the presence of straight rodlike particles, approximately 300 × 24 mμ, and other sinuous rodlike particles approximately 390 × 21mμ. The purified extracts from four young, apparently normal seedling orchid plants had no such forms. The particles are believed to be the infectious agents of a new virus complex of orchid, characterized by mottle symptoms of leaf and petiole.

Concentrated suspensions of the extracts from abnormal orchids showed marked streaming birefringence in the polarizing microscope. This property was absent in extracts from normal plants.

A pronounced Tyndall cone was shown in diseased extracts, not found in healthy preparations. Precipitin ring tests with serum from rabbits injected with dilute extracts from normal and diseased orchid plants gave a positive reaction, which was extinguished at a dilution of 1:30 of antigen from diseased stock. The extinction points in reactions with healthy extracts occurred at 1:5 and 1:10 dilutions of antigen.

The electron microscope is, therefore, an excellent instrument for the rapid diagnosis of plant viruses.

Battelle Memorial Institute
Columbus, Ohio

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RELATION OF AIR TEMPERATURE AND VIRUS CONCENTRATION TO MOSAIC RESISTANCE IN CABBAGE[1]

Glenn S. Pound

Selection for resistance to the mosaic disease (2) in the All Seasons and Hollander types of cabbage (*Brassica oleracea* var. *capitata* L.) has yielded lines that are uniformly highly resistant or highly susceptible. In a study of the progenies of crosses of resistant × susceptible lines it was found that resistance was incompletely dominant and polygenic in nature (7). The viruses (9) multiply in resistant plants, but in field plantings it has been observed that the expression of a certain degree of symptoms by infected plants is not always constant but varies somewhat with environment. The present study was undertaken to ascertain whether air temperature is related to symptom severity. Since it had been shown previously that the concentration of certain strains of turnip virus 1 in cabbage and horseradish was correlated with symptom severity (4, 6), the relationship of this factor to symptom expression in resistant cabbage also was studied.

MATERIALS AND METHODS.—Cabbage seed was sown in white quartz sand and seedlings were transplanted when in the cotyledonary stage to new sand in porcelain crocks, seven plants per crock. Plants were watered frequently with a balanced nutrient solution described by Hoagland and Snyder (1), each crock receiving an identical amount. Distilled water was used between feedings. When plants were in the second true leaf stage one crock of each variety was placed at each of four air temperatures, 16°, 20°, 24°, and 28° C., and inoculated with a mixture of cabbage viruses A and B (9). The mixture was used because the viruses usually occur together in nature to produce the mosaic disease. Sufficient trials with virus A were run to show that this virus alone responded to environment in resistant and susceptible plants exactly as it did when with virus B. The results reported herein refer only to the A component since the assay used was that for the local lesions produced by the A virus. All data given are for plants infected with virus A in combination with the B component, except for one trial in which the cabbage black ring virus (8) and a horseradish virus (3), both strains of turnip virus 1, were mixed with virus B and inoculated to the various lines for comparison with the cabbage A strain of turnip virus 1. In assays for the concentration of virus A, the youngest leaves were taken from each of the seven plants and a composite sample was triturated in a 0.1 M. sodium phosphate buffer of pH 7.0, usually at the rate of 1 gm. of tissue in 10 ml. Comparisons then were made in half-leaf inoculations to tobacco (*Nicotiana tabacum* L.) on which local necrotic lesions are produced. Carborundum was blown onto tobacco leaves with a DeVilbiss powder blower, and inocula were then applied with cheesecloth pads. In some tests, each comparison was made on eight to ten plants of three leaves each and analyzed independently of other comparisons.

When all possible comparisons of the four temperatures were desired, seven tobacco plants of three leaves each were used in an incomplete block design. The following arrangement was used; horizontal rows represent tobacco plants and vertical columns represent leaf positions on these plants:

A	B	C	D	E	F	G
B	D	F	E	G	A	C
C	F	E	A	B	G	D

Since the four temperatures allowed only six different comparisons, one treatment was duplicated each time. It can be seen that each treatment occurred once at each leaf position and once on the same plant with each of the other treatments. Usually twice the number of plants needed were inoculated, so that the half-leaf positions in which the inocula were applied could be reversed on half of the plants in order to eliminate any variability due to the inoculation stroke.

EXPERIMENTAL RESULTS.—*Relation of air temperature to resistance.*—As previously reported (5), the incubation period of mosaic in cabbage varied inversely with the air temperature within the range studied, being shortest at 28° and longest at 16° C. One line (83-366), which had been extremely susceptible in field plantings and which was included in all greenhouse tests as a susceptible control, also showed the same response of the incubation period to temperature. However, temperature did not seem to affect the ultimate severity of symptoms expressed in this line. After a sufficient lapse of time, usually 20–30 days, symptoms were about as severe at 16° as at 28° (Fig. 1, A).

Progenies 25-823, 25-839, 25-844, 25-849, 25-850, 25-852, 25-968, 25-969,83-502, 83-503, and 83-509 all showed moderate to high resistance in field tests (Table 1). In greenhouse tests they were almost completely resistant at 16° and 20° (Fig. 1), slightly to moderately susceptible at 24°, and moderately to highly susceptible at 28° C. At 28° most of the resistant lines developed mottling, stunting, and distortion as severely as did 83-366. Progenies 25-823 and 25-839, however, were considerably more tolerant at 28° than the others. In several trials 25-823 did not produce severe symptoms at 28°; 25-839 was only moderately susceptible at 28°.

On Jersey Queen, which was moderately susceptible

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in field trials, there was a gradient of symptom severity, symptoms being most severe at 28° and least severe at 16° with intermediate levels at 20° and 24° C. This gradient of symptom severity agrees with earlier studies made with this variety (5).

Two sister F_3 progenies (25-990 and 25-992) of a cross of a plant of susceptible 83-366 with a plant of resistant Wisconsin All Seasons showed field symptoms typical of the susceptible and resistant parents, respectively. In the greenhouse, 25-992 was highly resistant

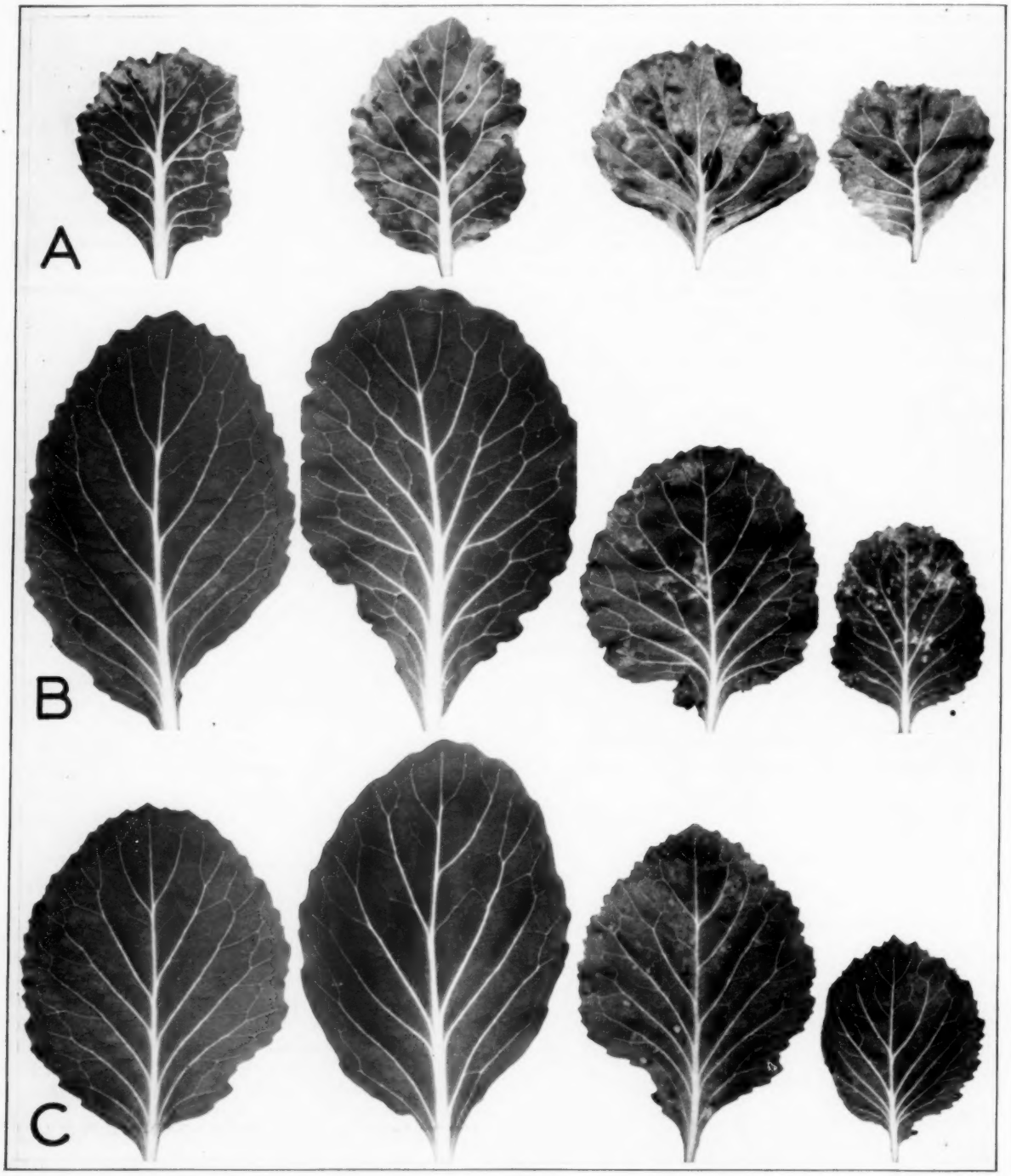

FIG. 1. Leaves from plants grown (left to right) at 16°, 20°, 24°, and 28° C. A, susceptible line 83-366; B, resistant line 25-844; C, resistant line 25-852. Note that 83-366 showed severe symptoms at all temperatures, whereas the two resistant lines showed symptoms only at 24° and 28°.

TABLE 1.—*Virus concentration in susceptible and resistant lines of cabbage grown at different air temperatures*

Variety or progeny	Average number of local lesions per half-leaf[a] with inoculum taken from plants grown at				Disease index in field planting[b]
	16° C.	20° C.	24° C.	28° C.	
Jersey Queen	7.5	21.0	58.9	114.6	51.0
83-366	6.6	11.4	17.6	22.8	76.7
25-823	0.1	2.1	5.0	3.3	12.8
25-839	0.9	0.8	4.6	43.1	7.9
25-850	0.5	1.9	2.0	181.4	11.6
25-969	4.1	9.2	16.9	161.8	9.6
83-502	6.5	15.8	46.1	130.1	19.4
83-509	4.2	3.3	6.3	104.4	20.1

[a] Values given are averages of nine half-leaf replications in assays made 30 days after inoculation. Comparisons should be made between temperatures for each variety.

[b] 0 = no symptoms; 20 = slight; 40 = moderate; 60 = severe; 80 = very severe.

TABLE 2.—*Relative virus concentration in susceptible and resistant lines of cabbage after growing for 30 days at 16°, 20°, 24°, 28° C. and then for 14 days at 28°, 24°, 20°, 16° C., respectively*

Variety or progeny	Average number local lesions per half-leaf with inoculum taken from plants grown at:			
	16° C.[a]	20° C.	24° C.	28° C.
Jersey Queen	4.4	6.6	22.7	26.4
83-366	10.0	9.0	10.6	19.0
25-823	0.1	0.6	0.4	4.8
25-839	0.2	0.4	2.8	12.2
25-850	0.0	0.4	1.1	20.4
25-969	0.1	0.3	2.7	12.1
83-502	0.6	4.2	13.8	32.3
83-509	2.8	5.1	9.7	37.0

[a] Plants at 16°, 20°, 24°, 28° had been grown at 28°, 24°, 20°, 16° C., respectively, for 30 days, at the end of which the assays given in table 1 were made. Values in table 2 are averages of nine half-leaf replications. Comparisons should be made between temperatures for each progeny.

at 16° and highly susceptible at 28°; progeny 25-990 showed severe symptoms at all temperatures.

In one experiment involving six resistant lines, line 83-366, and Jersey Queen, all infected plants were allowed to grow at the different temperatures for 60 days when plants at 16°, 20°, 24°, 28° were transferred to 28°, 24°, 20°, and 16° C., respectively. New growth of the plants at each temperature rapidly showed symptoms previously shown for that specific temperature, and a complete reversal in symptoms had occurred after 30 days. Line 83-366, however, showed little response to reversal in temperatures. In figure 2 are leaves taken from progeny 25-969 before and after temperature reversal.

When the cabbage black ring virus (8) or the horseradish isolate (HR41) of turnip virus 1 was mixed with virus B and inoculated to the progenies described above, almost identical reactions to those described for viruses A + B were observed. Progenies resistant to the A + B combination at 16° were also resistant to the other combinations at 16° C. Also, as with the complex containing virus A, these resistant plants developed rather severe symptoms at 28° C. Progenies 25-823 and 25-839, however, as with virus A, remained quite tolerant at 28°. More necrosis developed at 16° with the black ring and HR41 isolates than with the cabbage A isolate.

Relation of virus concentration to resistance.—With every resistant line tested, there was a close correlation between symptom severity and virus concentration. In table 1 are the results of one experiment in which line 83-366, Jersey Queen, and six resistant lines at each of the four air temperatures were assayed for virus concentration according to the incomplete block design for assays reported in an earlier similar study (4). At 28° C. where symptoms for all lines except 25-823 and 25-839 were moderate or very severe, the assays showed virus concentration to be very high; and at 16° and 20° C. where the resistant lines showed few or no symptoms, the virus concentration was very low. With most of the resistant lines, resistance was very high at 16° and 20° but broke somewhat at 24°; breakdown was complete at 28°. However, lines 25-823 and 25-839 showed only moderate symptoms even at 28° and had a corresponding low level of virus concentration at that temperature. Lines 83-509 and 25-850 showed almost complete resistance and had a corresponding low level of virus concentration at 24°.

Temperature responses were different in Jersey Queen and line 83-366 which were used as checks. Jersey Queen showed a gradient of virus concentration, which increased from low to high temperature and corresponded closely with the gradient of symptom severity. This has been previously shown for this variety (5). Line 83-366 was extremely susceptible and the number of local lesions obtained in assays did not coincide with the severity of symptoms. It is thought that some inhibitory action on the virus might possibly occur when leaves from this line are macerated, for it is the only line where symptom severity has not been closely correlated with virus concentration. Symptoms for this line are ultimately as severe at low temperature as at high temperature (Fig. 1, A). A gradient of concentration like that in Jersey Queen occurred in assays soon after inoculation, but ultimately the concentration differed only slightly from low to high temperatures; in some experiments a higher concentration was found at 16° than at 28° C. This is the only line of cabbage tested to date that has shown this temperature reaction.

In another experiment, several resistant lines and susceptible 83-366 were grown at 16° and 28° C. and when assays were made each lot was compared with 83-366 at both temperatures. In these assays the number of local lesions with inoculum from each of the resistant lines at 16° was much less than with that from 83-366 at 16°, but inoculum from 28° was as high as or higher than that from 83-366 at 28°. Also, when

28° and 16° temperatures were compared for each line, many more local lesions were obtained with inoculum from 28° than with that from 16°. Included in these tests were the two sister F_3 progenies (25-990 and 25-992) of a cross between plants of susceptible 83-366 and a resistant line of Wisconsin All Seasons. In symptom expression progeny 25-990 was very similar to the susceptible parent, whereas 25-992 closely resembled the resistant parent. Assays showed a corresponding picture of virus concentration; 25-992 had high concentration at 28° and almost none at 16°; little difference occurred between the two temperatures for 25-990.

Immediately after the assays in table 1 were made, plants were shifted from 16°, 20°, 24°, and 28° to 28°, 24°, 20°, and 16° C., respectively. After 14 days' incubation, assays were made again from the new growth. The results (Table 2) showed that with reversal in symptoms there was a corresponding reversal in virus concentration. However, the virus concentration at 28° was considerably lower for all lines than in the previous assays of plants at this temperature. Several lines were kept an additional two weeks and assays then indicated higher virus concentration.

All of the above experiments were repeated several times during a 3-year period and similar results were obtained each time.

Several tests were made of resistant lines inoculated with virus A alone. In every test the resistant lines showed few or no symptoms at 16° but moderate to severe symptoms at 28°; the virus concentration was very low at 16° and very high at 28° C.

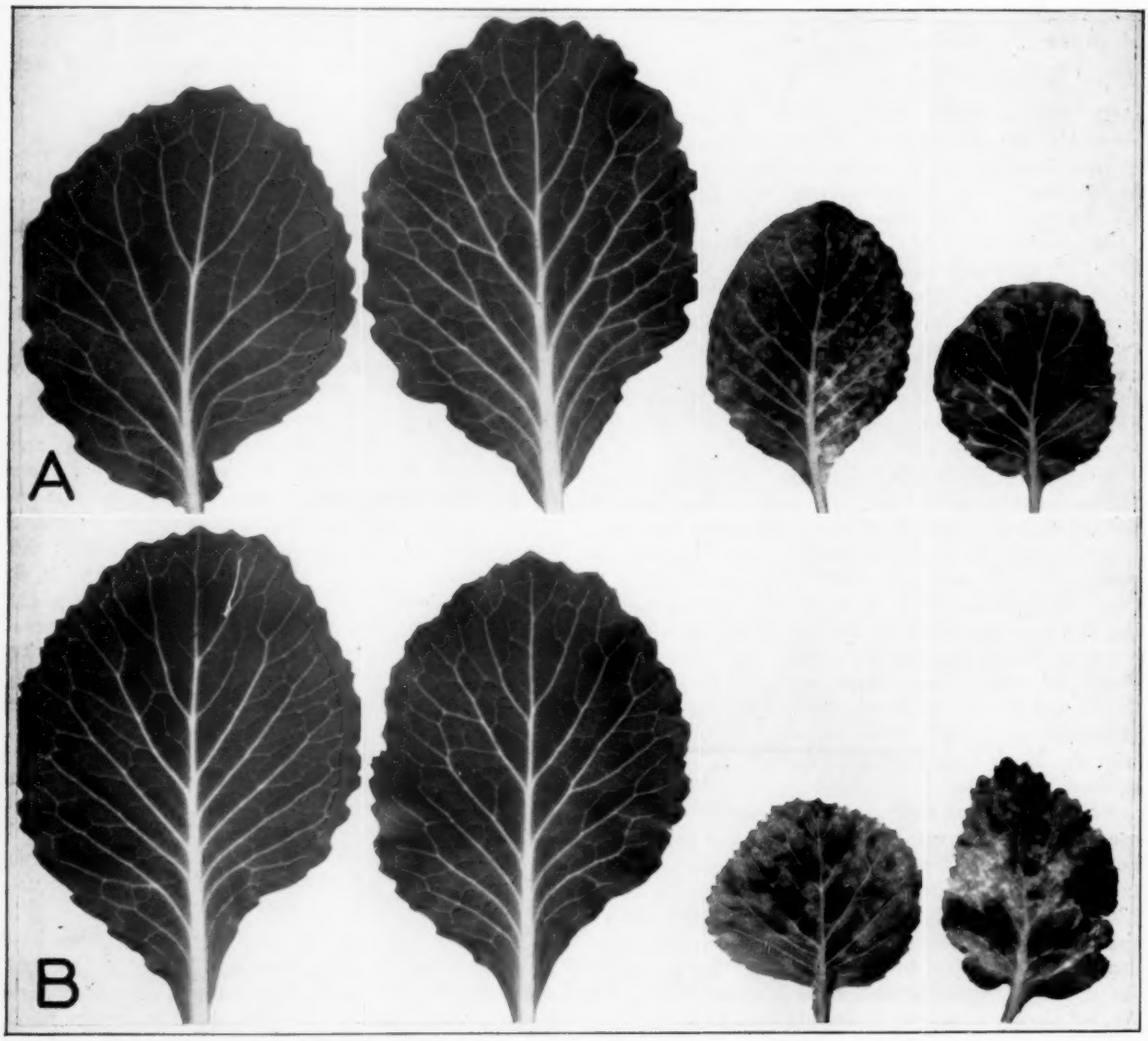

FIG. 2. Leaves from mosaic-infected plants of resistant 25-969 grown at 16°, 20°, 24°, and 28° C., left to right, respectively. A, from plants grown for 60 days at their respective temperatures; B, from same plants 30 days after temperatures were reversed to 28° 24°, 20°, and 16° C., left to right, respectively. Note that reversal of temperature resulted in reversal of symptoms.

Concentration assays were not made for the cabbage black ring or horseradish viruses.

A test was made to determine whether cabbage leaves from resistant lines grown at 16° C. contained some inhibitor not present in leaves from the same lines grown at 28°. To aliquots of inoculum taken from *Nicotiana glutinosa* L. were added equivalent amounts of extract of healthy leaves of resistant lines grown for 30 days at 16° and at 28° C. Other aliquots of the virus inoculum were diluted to the same extent with a neutral buffer for a standard check. Each sample containing cabbage tissue was compared with the standard check in half-leaf inoculations to tobacco. The standard inoculum had 1 gm. of infected tissue in 10 ml. and the other treatments had an identical amount of infected tissue plus 1 gm. of cabbage tissue in 10 ml. The results obtained with two lines (25-844 and 25-969) indicated that resistance could not be explained on the basis of an inhibitor. In progeny 25-844, the addition of cabbage sap from plants grown at 28° to the standard inoculum resulted in a reduction of 69.7 per cent in the number of local lesions; the addition of sap from plants grown at 16° to the standard inoculum reduced the number of local lesions by 65.5 per cent. With progeny 25-968 the reductions were 50.1 and 48.4 per cent for 28° and 16°, respectively.

Discussion.—In a previous paper (7) it was shown that resistance to the cabbage A component of the mosaic disease did not necessarily function as such for the B virus component. Although most resistant lines are markedly resistant to both viruses, some that are resistant to the virus A component develop severe late-season chlorosis from infection with the B virus (7). In these studies the presence of the B virus did not alter the relationship of virus A to the expression of resistance, since the same effects were obtained with virus A alone as in combination with virus B. Although the symptoms of the two viruses together are more severe than those of either virus alone (5), especially at high temperatures, it is believed that the virus A component is much more important in the symptom syndrome than virus B. It also is believed that certain genes which function for resistance to virus A must also function for resistance to virus B, because symptoms of the latter virus in resistant lines are much milder than in susceptible varieties such as Jersey Queen. The genes governing the expression of the late-season chlorosis induced by virus B must be independent of those related to susceptibility to other phases of the disease.

Data herein show that the resistant lines of cabbage readily become infected with virus A and would be expected to develop moderate to severe symptoms only when mean daily temperatures exceed 80° F. Since this seldom happens in cabbage-growing areas, this breakdown of resistance at high temperatures is of little practical importance. It explains, however, slight seasonal fluctuations in symptoms expressed by resistant selections. It is interesting that the temperature at which this polygenic resistance becomes nonfunctional is very close to that at which the polygenic resistance to Fusarium yellows also becomes nonfunctional.

The relation of air temperature to expression of resistance is, in fact, the relation of air temperature to the activity of the virus in the host. High virus concentration in the resistant lines was almost always correlated with severe symptoms. When temperatures were reversed so as to bring about a reversal in symptoms, a corresponding reversal in virus concentration occurred. All but one or two of the resistant progenies tested showed severe symptoms and high virus concentration at 28° and few or no symptoms and low virus concentration at 16°. Progeny 25-823, however, in several trials never developed severe symptoms at 28° and assays always indicated a very low level of virus concentration. Whether 25-823 has some factors governing its response to temperature other than those in sister lines or whether it may have a greater number of resistant genes is not known. Jersey Queen, a moderately susceptible variety, showed a gradient of virus concentration corresponding with the gradient of symptom severity at the different temperatures. Line 83-366, however, an extremely susceptible line at all temperatures, never had a virus concentration comparable to the severity of symptoms.

SUMMARY

Progenies of cabbage highly resistant to the mosaic disease have expressed this resistance under artificial inoculation only at 24° C. or below. At 28° C. infected plants develop severe symptoms. Assays of concentration of the cabbage A component of the causal virus complex indicated a close correlation between symptom severity and virus concentration. At 16° and 20° C., where resistance was very high, the virus was present in very low concentrations; at 28°, where symptoms were severe, the virus concentrations were very high. Intermediate reactions usually ocurred at 24°. Reversing the air temperatures from 16°, 20°, 24°, and 28° to 28°, 24° 20°, and 16°, respectively, led to corresponding changes in symptoms and virus concentrations.

Department of Plant Pathology, University of Wisconsin
and
U. S. Department of Agriculture
Madison, Wisconsin

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BLUEBERRY GALLS CAUSED BY A STRAIN OF AGROBACTERIUM TUMEFACIENS[1]

J. B. Demaree and N. R. Smith

Galls on the aerial portions of blueberry plants have been known for many years. Although they are of minor importance in cultivated fields, they have been observed frequently in nurseries. In one nursery in New Jersey visited by the senior author, they were sufficiently abundant to cause the owner considerable apprehension.

This paper reports the results of observations of blueberry galls and studies extending over several years, and the isolation and description of the causative organism.

OCCURRENCE AND DESCRIPTION OF THE GALLS.—One of the earliest records of galls on blueberries was a photograph taken by the late F. V. Coville in 1914 in the greenhouses of the U. S. Department of Agriculture, Washington, D. C. Later, Brown(2), in a study of the etiology of tumors on a number of woody plants, including blueberry, was unable to isolate a bacterium pathogenic to blueberry, but by long sterilization of the gall surfaces with 1:1000 mercuric chloride she obtained many pure cultures of the fungus *Phomopsis*. Because inoculation of the blueberry with this fungus was said to cause galls, the disease has been called the "phomopsis gall" since that time.

In the present work, galls were collected in New Jersey, New York, Michigan, Washington, and British Columbia. Usually they were on branches and small twigs, but sometimes they occurred at the base of the canes near the ground line. None has ever been observed on the root system. Although the gross appearance of the galls varied somewhat, they usually were black or dark brown, rough, and hard. They formed as single globose structures pea-size and larger, or as globose or elongated excrescences due to one gall growing into another. Sometimes the entire circumference of the shoot was involved. Galls from Washington and British Columbia were of the elongated form (Fig. 1, C) more often than those in the East.

That the causative organism is perennial is indicated by new galls that sometimes break through the cortex either above or below the old ones formed during the previous growing seasons, suggesting that the organism has spread along the cambium or sap-conducting tissues. Affected branches and twigs often die. No evidence of bud proliferation has been observed on this type of gall. This lack of proliferation distinguishes it from the blueberry tumor caused by a species of *Actinomyces*, as reported by Demaree(3).

THE BLUEBERRY ORGANISM.—*Isolation and pathogenicity.*—In 1946 the senior writer collected in a badly affected nursery in New Jersey several galls which appeared to be identical with the so-called phomopsis galls. Some of the young outgrowth was crushed and used to inoculate succulent blueberry seedlings by the needle-prick or incision method. In about 10 days, swollen tissues developed around each inoculation and eventually typical galls resulted. This crude experiment showed that the infective agent was readily transmissible.

An attempt was then made to isolate pathogenic organisms from young galls. The surfaces of the galls were sterilized with 1:1000 mercuric chloride and pieces of tissue were cut aseptically from the inside and planted on nutrient agar slopes. Growth occurred in most of the tubes and six were selected as being representative. Of these, four were fungi and two were bacteria. Although admittedly the cultures probably were not pure, each of them was inoculated into young blueberry plants. None of the fungi produced galls but both bacterial cultures, BCG5 and BCG6, did. The latter were plated in the usual manner and three colonies of BCG5 and two (one yellow and one white) of BCG6 were selected for further testing for pathogenicity. Only BCG5Y and the white strain of BCG6 caused galls (Fig. 1, A). Subsequently another pathogenic bacterial culture, W6, was isolated from a gall on a blueberry plant grown in Washington. This culture differed from the others in certain minor respects.

Specificity.—Over about 5 years a large number of blueberry plants were inoculated with culture BCG5Y. Cultures BCG6 and W6 also were used to some extent, but since there were no essential differences in pathogenicity among the three cultures, only one was used in most tests, in order to reduce the amount of work; an adequate number of inoculations was always made and controls were kept at all times. Occasional failure of gall development following inoculation with the pathogen might be attributed to the use of plants in

[1]Accepted for publication October 6, 1951.

TABLE 1.—*Pathogenicity of the blueberry-gall organism and of apple and peach strains of the crown-gall bacterium, as indicated by development of galls on several plant genera including blueberry*

Plant inoculated	Gall development[a] when inoculum was from:		
	Blueberry	Apple	Peach
Aster	?	+++	++++
Azalea	+	0	0
Blueberry	++++	0	0
Castor bean	+	+++	+++
Impatiens	0	+	—
Kalanchoe	0	+++	++++
Olive	0	0	0
Peach	++	++++	++++
Raspberry	+	+	+
Tomato	+	+++	+++
Vinca	0	+++	—

[a]Symbols indicate the development of galls as follows: o, none; ?, questionable; +, very slight; ++, slight; +++, moderate; ++++, extensive; —, no inoculations made.

the hardwood stage, or to some error in manipulation. Since a large number of inoculations was made over a long period, only a summary of the results is given.

Since a preliminary survey of the cultural characters of the blueberry gall organism suggested that it might be related to, if not identical with, the crown gall bacterium, *Agrobacterium tumefaciens* (Smith & Townsend) Conn (1), two strains of the latter[2] were used in parallel inoculations on adequate numbers of several plants. The results show (Table 1) that blueberry was the favorite host for the blueberry organism, only slight gall formation taking place on peach, very slight on azalea, castor bean, raspberry, and tomato, and questionable or none on aster, impatiens, kalanchoe, olive, and vinca. In comparison, the strains of *A. tumefaciens* were very active, gall formation being moderate or extensive on aster, castor bean, kalanchoe, peach, tomato, and vinca, very slight on raspberry and impatiens, and absent on azalea, blueberry, and olive.

Galls formed on blueberry plants only when the rapidly growing shoots, young leaves, and buds were injured and then inoculated with the pathogen. Excised shoots inoculated at the stub end formed galls (Fig. 1, B); this suggested that the organism may be spread in the field or nursery when the plants are pruned or softwood cuttings are made. Spraying with a suspension of the organism was without effect.

Of special interest was the lack of any reaction of kalanchoe to the blueberry organism, the wounds made at the time of inoculation having healed promptly (Fig. 2, C). Both strains of *A. tumefaciens*, however, caused large tumors (Fig. 2, A, B).

Numerous unsuccessful attempts were made to cause galls on blueberry by inoculating it with the apple and peach strains of *A. tumefaciens*. This corroborates

[2]Apple strain, No. 671, isolated by E. A. Siegler, formerly pathologist, U. S. Department of Agriculture; and peach strain, No. 176, in the junior writer's collection, from N. A. Brown in 1928.

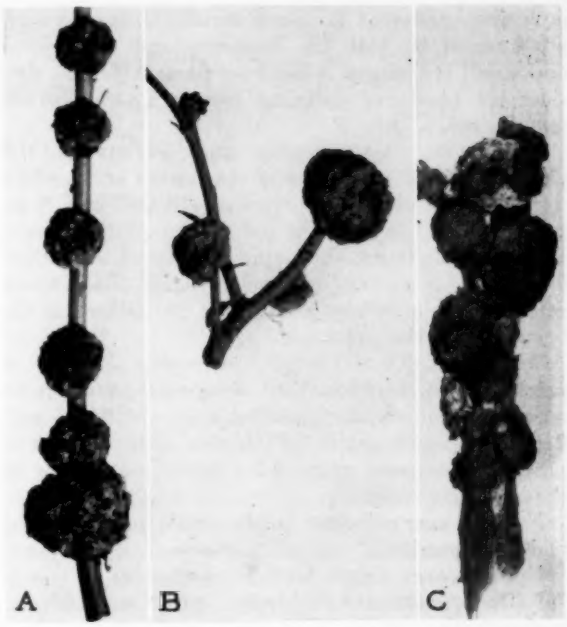

FIG. 1. Galls on blueberry caused by *Agrobacterium tumefaciens*. A, artificial infection with culture BCG5Y. B, ends of excised shoots inoculated with isolate shown in A. C, natural infection.

the results obtained by Brown (2), who failed to infect blueberry with strains of the crown-gall pathogen from peach, raspberry, hop, and dahlia.

The galls on blueberry caused by the blueberry pathogen highly resemble abnormalities induced by the crown-gall organism on certain other plants. The similarity of the cultural characteristics, together with the differences in pathogenicity (Table 1), seems to suggest that the isolate is either a strain of *A. tumefaciens*

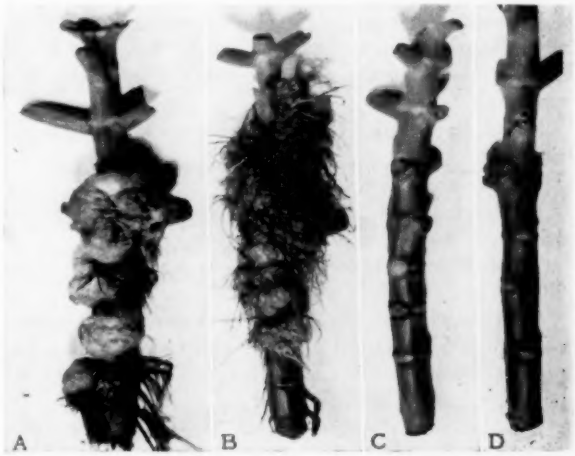

FIG. 2. Results of inoculating stems of kalanchoe (A) with *Agrobacterium tumefaciens*, apple strain No. 671; (B) with *Agrobacterium tumefaciens*, peach strain, No. 176; and (C) with the blueberry organism BCG5Y. D, control; injured but not inoculated.

or a new variety of it. Since certain hosts, however, are affected by both the blueberry and the typical crown-gall organisms, it has been thought best to consider the blueberry pathogen merely a strain of *A. tumefaciens.*

Description.—A comparative study was made of the morphology and physiology of the eastern and western strains of the blueberry organism (BCG5Y and W6) and of two strains (apple and peach) of the typical crown-gall organism. In certain characters there were disagreements among the four cultures. Such characters are indicated by an asterisk (*) placed at the beginning of the paragraph.

* Rods: 0.3–0.6 × 1.5–3.0 μ, occurring singly or in pairs, old cells very granular. Capsules demonstrable on potato-peptone-yeast-mannitol agar. Motile, 1 to 3 flagella. Gram negative. (The rods of the apple and peach strains were about 0.2 μ wider than those of the blueberry strains.)

Nutrient agar colonies: Small, round, not distinctive, and slow-growing.

Nutrient agar slants: Growth slow, poor.

* Glucose-nutrient agar slants: Growth scant. (Abundant growth with the apple and peach strains.)

Glucose-nitrate agar slants: Growth abundant, heaped.

* Glucose-asparagin agar slants: Good ribbon growth, acid produced. (An alkaline reaction was produced by the apple and peach strains.)

* Soybean agar: Growth abundant, raised, and soft. (Eastern strain did not grow.)

Potato-peptone-yeast-manitol agar: Abundant, white, soft, and raised growth.

Potato: Growth scant, not distinctive.

* Milk agar plate: Very abundant, heaped, and slimy growth in 10 days; moderate zone of hydrolysis of the casein. (Eastern strain did not hydrolyze the casein, although milk in tubes was slowly digested.)

Reduction of nitrate to nitrite: Negative. (Sometimes a weak nitrite test was obtained.)

Fermentation reactions: With ammonium phosphate as the source of nitrogen, acid, but no gas, from arabinose, xylose, glucose, lactose, sucrose, glycerol, and mannitol.

Starch hydrolysis: Positive; narrow zone of hydrolysis.

Citrate utilization: Positive.

Production of acetylmethylcarbinol: Negative. (Scant, if any, growth in the broth.)

Temperature relations: Optimum temperature about 28° C.; no growth at 37° C.

* Absorption of Congo red: Positive. (On potato-peptone-yeast-mannitol agar, Congo red was absorbed poorly by both blueberry strains, but very well by the peach and apple strains.)

* Vitamins and amino acids[3]: Growth in a vitamin-free basal medium (4). (Eastern strain required biotin and nicotinic acid for growth. A better growth resulted if amino acids were added also.)

Discussion.—The nutritional requirements of the eastern strain of the blueberry pathogen resembled those of *Agrobacterium rubi* (Hildebrand) Starr & Weiss, except that pantothenate was not required. The western strain, on the other hand, was like *A. tumefaciens.* This situation is confusing and can be clarified only by a more intensive study of numerous isolates from blueberry galls and comparison of these with a like number of strains of *A. rubi.* Starr (4) has "never been convinced that a difference in nutritional requirements alone is a good way to separate species of bacteria." The writers agree with him and do not, therefore, attach any real importance to the difference in the vitamin requirements of the two isolates, but place emphasis on the ability to form galls on blueberry. Whether additional isolates from similar sources would have the same characteristics as the blueberry strains studied cannot, of course, be stated. Due consideration must always be given to the factor of variation among strains of any species of bacterium.

[3]M. P. Starr, University of California College of Agriculture, Davis, California, kindly made these determinations, for which the writers are very grateful.

SUMMARY

Two strains of a bacterium from galls on blueberry, thought to be identical with those attributed to the fungus *Phomopsis,* were isolated and studied. Although slightly different from each other physiologically, the two strains may be considered to belong to the same species.

The blueberry organism repeatedly caused large galls when inoculated into the meristematic tissue of blueberry plants but consistently failed to cause galls on kalanchoe, and gave questionable results on aster. Two strains of *Agrobacterium tumefaciens* (apple and peach), on the other hand, failed to cause overgrowths in blueberry but produced large excrescences on kalanchoe and aster.

Culturally, the blueberry organism resembles rather closely the apple and peach strains of *A. tumefaciens.* This, together with the fact that all three strains produced small galls on certain plants, leads to the conclusion that they should be considered strains of the same species.

No evidence was found that blueberry gall is caused by the fungus *Phomopsis.*

Plant Industry Station
Beltsville, Maryland

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THE PRODUCTION AND ROLE OF ANTIBIOTICS IN THE SOIL. II. CHLOROMYCETIN [1]

David Gottlieb and Paul Siminoff [2]

The theoretical and practical significance of the production and activity of antibiotics has been discussed in the initial paper of this series (13). Our previous studies have indicated that because of the diverse chemical structures of such materials, generalizations on this question must be postponed until the relationship between many antibiotics and the soil has been elucidated (2, 3, 12, 13). Apparently antibiotics can be divided into three groups on the basis of their ionic dissociation: 1) basic antibiotics, compounds that are strongly bound and inactivated by the soil complex, e.g., streptomycin and streptothricin; 2) neutral antibiotics, compounds that are weakly bound and retain a relatively high degree of activity in the soil, although considerably less than in nutrient broth, e.g., chloromycetin and actidione; and 3) acidic antibiotics, compounds that, particularly when in the form of the acid ion, fall in an activity range in the soil between that of the basic and neutral antibiotics, e.g., clavacin. This is not a rigid classification, however, since some materials will have both basic and acidic properties and the proportion of the functional groups will determine the final properties. Furthermore, their dissociation would be affected by the hydrogen-ion concentration of the soil. Another feature which must be considered is the effect of the normal soil microflora on the antibiotic. Such populations could, as in the case of streptomycin, decompose the antibiotic and thus vitiate the inhibitory effect of its production on the soil microflora (10).

The investigations reported in this paper deal with the production and fate in soil of the neutral antibiotic, chloromycetin. This substance is synthesized by the soil-inhabiting actinomycete *Streptomyces venezuelae*, and is active against many bacteria, both Gram negative and Gram positive. The structure of chloromycetin has been identified as D(—)threo-1-p-nitrophenyl-2-dichloracetamido-1,3-propanediol (11). The existence of an aromatic nitro group in the molecule makes this antibiotic unique among compounds naturally produced by living organisms and has been extremely useful in the identification and quantitative determination of the substance in complex systems such as soils.

Preliminary studies have shown that, unlike the basic antibiotic streptomycin, chloromycetin is not greatly inactivated in soil. The antibiotic can be extracted from soil after it has been adsorbed. Therefore, if chloromycetin is produced in the soil, it should be detectable by chemical and biological methods. Furthermore, if sufficient quantities are produced in soil, this production should be reflected in the decline of a susceptible microorganism. As seen in laboratory studies, the synthesis of this antibiotic depends upon the type of nutrients supplied. The addition to the soil of organic and mineral fertilizers, a common agricultural practice, might stimulate antibiotic formation. However, the very large and highly diversified population of microorganisms in normal soils could, conceivably, hinder the growth of the antibiotic producer. Important, too, is the question whether chloromycetin can remain unchanged in the presence of this diverse microflora, for, even if produced, it might be metabolized by some of the bacteria, fungi, or actinomycetes. Finally, the presence of chloromycetin in soil could give rise to changes in the composition of the soil's microflora which might upset its prevailing biodynamics. These viewpoints formed the bases for the studies on the production and role of chloromycetin in soil.

METHODS.—Soil competition studies involving the effect of *Streptomyces venezuelae* on *Bacillus subtilis* in sterile unamended soil were carried out in substantially the same way as in the first paper of the series (13). Of the many organic solvents tested, methanol proved the most efficient for recovering chloromycetin. Soils containing water at 60 per cent of their moisture-holding capacity were extracted three times on a reciprocal shaker with 25 ml., 15 ml., and 10 ml. of methanol, for 25 min., 15 min., and 10 min., respectively. The extracts were then filtered under suction through two layers of filter paper (Whatman No. 1) on a Buchner funnel and the residues washed three times with methanol. The filtrates were then evaporated to dryness in a forced-draft oven at 70° C. Finally the dry residue was taken up in 5 ml. of 10 per cent methanol and assayed for chloromycetin. This elution technique recovered 70 to 80 per cent of the antibiotic contained in the soil and will be referred to as the methanol-wet soil method.

Procedures used to determine chloromycetin in the soil extracts were: 1) A bioassay with *Pasteurella avicida* as test organism; seeding medium, Difco brain-heart infusion broth containing 1.5 per cent agar, adjusted to pH 6 with 10N HCl; detecting 2 to 10 μgm. chloromycetin per ml. 2) A bioassay with *B. subtilis* as test organism; seeding medium, nutrient agar adjusted to pH 6; detecting 10 to 100 μgm. per ml. (Standard paper-disc methods were used for both bioassays.) 3) A chemical assay (colorimetric determination) adapted by us to the soil studies, fol-

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[2] Respectively, Associate Professor of Plant Pathology, Department of Horticulture, and University fellow in soil microbiology, Agronomy Department, University of Illinois, Urbana, Illinois. The authors wish to acknowledge the helpful suggestions of Dr. O. H. Sears in this research.

lowing the method of Bessman and Stevens but omitting protein precipitation; detecting 2 to 100 μgm. per ml.

To account for the long delay in the appearance of chloromycetin in the mixed cultures, the effect on chloromycetin of a large population of *B. subtilis* in soil was studied. One ml. of a washed suspension of *B. subtilis* was added to each of a number of flasks containing 20 gm. of sterile clay-loam soil. The flasks were then incubated for 18 days at 28° C., to obtain a population of cells of the bacillus nearly equivalent to the numbers found in the competition studies. Duplicate flasks were plated out on both plain Difco nutrient agar and agar containing 10 μgm. of chloromycetin per ml. This gave the population density in the soil and also the number of cells resistant to at least 10 μgm. per ml. of the antibiotic. Of the remaining flasks, two were set aside as growth controls of *B. subtilis*. Flasks in one set were autoclaved for 1 hr. to kill the bacterium; the others retained the viable cells. The flasks were then divided for treatment with 10 μgm. and 15 μgm. of chloromycetin per gm. of soil. Three flasks from each treatment were immediately extracted for determination of recoverable antibiotic at 0 days. All soils were incubated for 8 days after the addition of the chloromycetin and then analyzed for the antibiotic and for the number of cells of *B. subtilis*.

To study the effect of the microflora in normal air-dried, dark prairie soil, the antibiotic was added in 20-gm. amounts to 125-ml. Erlenmeyer flasks; it was brought to optimal moisture content and incubated for 12 days to develop an active soil population and the numbers of bacteria, actinomycetes, and fungi were then determined by plate counts. Sodium asparaginate-glucose agar was used as the plating medium for the actinomycetes and bacteria.[3] Immediately after autoclaving, 100 μgm. of actidione per ml. of the agar were added, to suppress the fungi (9). The soil fungi were counted on standard peptone-glucose agar to which was added rose bengal to a final concentration of 1:15,000 (6). The number of chloromycetin-resistant organisms was also determined by plating the soils on the appropriate agar amended with 10 μgm. of chloromycetin per ml. Obviously the concentration of the antibiotic selected was purely arbitrary and was not an absolute standard of resistance. Population changes in both the treated and untreated soils were estimated at 0-, 7-, and 14-day intervals.

A number of flasks was left untreated as soil controls. Half of the remaining flasks were autoclaved to provide sterile controls. To these sterile controls and the nonsterile soils were added 50 μgm. of sterile chloromycetin per gm. of soil. The soils were analyzed for recoverable chloromycetin by both chemical and biological assays at the end of 0, 1, 3, 7, and 14 days. By this technique it is possible to detect intermediate breakdown products of chloromycetin, for the bioassays determine the total active antibiotic and the colorimetric assay determines both aromatic nitro and amine compounds. The value for reducible nitro compound minus that for total active chloromycetin measures the inactive nitro fragments. A decrease in active antibiotic and a corresponding appearance of aryl amines indicated a reduction of the antibiotic to the corresponding amine.

[3] The medium consists of: sodium asparaginate, 1.0 gm.; glucose, 1.0 gm.; $(NH_4)_2SO_4$, 1.0 gm.; KH_2PO_4, 1.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; $CaCl_2$, 0.1 gm.; $FeCl_3$, trace; agar, 20 gm.; distilled water, 1000 ml. The medium is adjusted to pH 6.8 with 10N NaOH.

RESULTS.—*Interaction of chloromycetin with clays and soils.*—Concentrations of chloromycetin as high as 25 μgm. per mgm. of clay did not flocculate either a bentonite or an illite clay, nor was adsorption indicated by an alteration of the light-transmitting power of bentonite under such conditions (12). Between concentrations of chloromycetin of 5 and 25 μgm. per mgm. the maximum adsorption by either clay was about 1 μgm. per mgm. An X-ray diffraction picture of a chloromycetin-treated bentonite showed no spread of the crystal lattice, the clay treated with the antibiotic showed an 001 spacing of 12.4 Å as compared with 13.0 Å for the untreated clay. In light of this evidence it is probable that even though a small amount of the antibiotic could be adsorbed by clay, a valency bonding did not take place.

The picture presented by the adsorption of chloromycetin by a dark, heavy prairie soil is somewhat obscure. If we assume a clay loam contains at least 20 per cent clay, then 1 gm. of the soil contains 200 mgm. of clay. The predominant clay type of the soils in the Illinois area is illite, which will remove from aqueous solution 1 μgm. of chloromycetin per mgm. of clay. Therefore, if 1 gm. of soil contained 200 mgm. of illite it should remove 200 μgm. of chloromycetin from solution. But this was not the case in actual test. At concentrations in aqueous solution as low as 5 μgm. per gm. of soil, small amounts of chloromycetin could be recovered after such solutions of the antibiotic were shaken with soil for 2 hr. (Fig. 1). Recovery was between 5 and 20 μgm., a little more than 20 per cent. At concentrations greater than 20 μgm., recovery was about 35 per cent. Apparently, the clay fraction is not a particularly important factor in chloromycetin uptake by soil.

Over the general range of concentrations of chloromycetin from 5 to 100 μgm. per gm. of soil, 50 per cent methanol will elute approximately 65 per cent, and the methanol–wet-soil procedure from 70 to 75 per cent, of the antibiotic added. Since 35 per cent is recoverable from water, the difference between the amount actually eluted and the amount which could be recovered from water represents the extraction efficiency of the two solvent systems (Fig. 1). For example, at 100 μgm. of chloromycetin per gm. of soil, 50 per cent methanol will extract about 46 per cent of the antibiotic adsorbed from aqueous solution and the methanol–wet-soil procedure will extract 54 per cent of the adsorbed antibiotic.

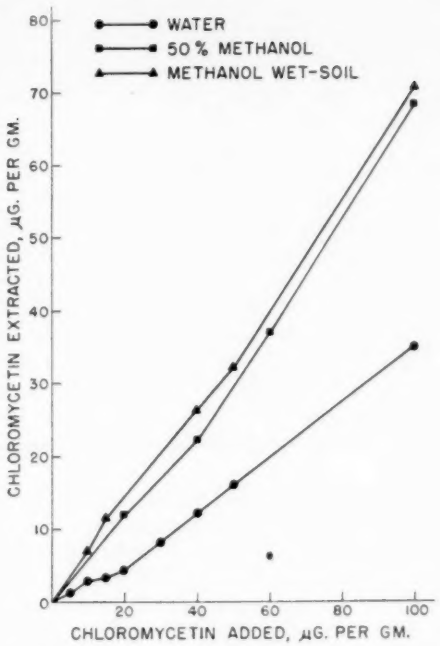

FIG. 1. The extraction of chloromycetin from soil.

Chloromycetin, when added to soil, inhibits the growth of *B. subtilis*. Two weeks after the bacteria were added to control soils they had multiplied to 27×10^5 viable cells per gm. of soil. Under similar conditions, at concentrations of 5 and 10 μgm. of chloromycetin per gm. of soil the populations were 11.4×10^5 and 9.3×10^5 respectively; these differences are statistically significant at the 1 per cent level. Growth is prevented entirely at 15 μgm. per gm. of soil. Since the soil had been air dried, the 5 ml. of water which had been added to each 20-gm. sample can be considered as free water, i.e., not bound. The 5 ml. of water contained 300 μgm. of chloromycetin when the antibiotic was added at the level of 15 μgm. per gm. of soil. This is the minimum required to prevent the growth of *B. subtilis* in soil. Therefore, in terms of soil solution the concentration is 60 μgm. of chloromycetin per ml. of soil solution. *B. subtilis* is usually inhibited by 1 μgm. of chloromycetin per ml. of a nutrient broth, but in the soil 60 μgm. per ml. were required; the bacterium was 60 times less sensitive in soil than in broth. Thus, even in the case of a neutral antibiotic which is not greatly adsorbed, the soil exerts a considerable protective effect.

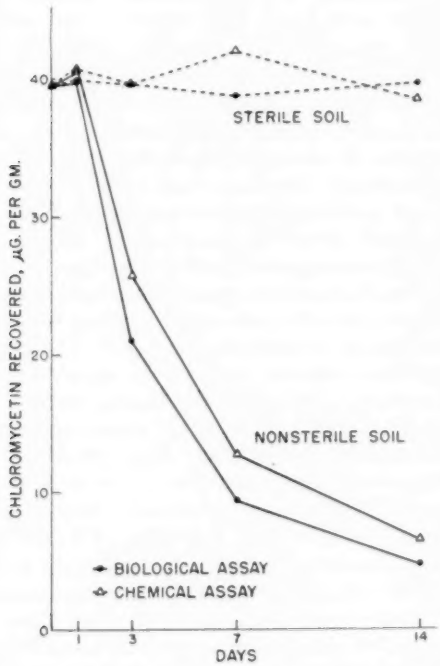

FIG. 2. The degradation of chloromycetin in a "normal" soil. Initially 50 μgm. per gm. of soil were added.

Production of chloromycetin in soil.—Although several instances of antibiotic formation in soils have been reported, the formation of such materials was dependent upon the addition of various supplements to the soils. No data were obtained for their production in normal soils. Another important factor is the lack of evidence for the identity of the inhibitory material in the soil with the known antibiotic (4, 8). This inability to demonstrate the production of antibiotics in untreated soils might lead to the conclusion that antibiotic formation is not a feature of the normal metabolic patterns of the microorganisms, but that it is rather an aberrant phenomenon, occurring on enriched substrates but not under the prevailing nutritional environment of normal soil.

The data in tables 2 and 3 show that *S. venezuelae* is capable of producing an antibiotic in soil which had been sterilized previous to its infestation with the actinomycete. This material was produced even in the presence of at least one other organism, the sensitive bacterium *B. subtilis*. Chloromycetin was detected in soil 1) when the actinomycete was added to soil 14 days prior to, 2) simultaneously with, and 3) 14 days after addition of the bacterium. The amounts of antibiotic extracted varied from 0.5 μgm. per gm. of soil to slightly more than 1.0 μgm. When only *S. venezuelae* was present the antibiotic was always detected much earlier than in the mixed cultures.

Competitive effects between S. venezuelae and B. subtilis in soil.—Whether introduced into the soil cultures before, simultaneously with, or after *S. venezuelae*, *B. subtilis* was not seriously inhibited (Table 1). When introduced simultaneously with the actinomycete, the bacterium grew relatively slowly during the first 36 days of incubation; growth in mixed culture was about 60 per cent of that in the control which contained only *B. subtilis*. Nevertheless, the number of viable cells increased steadily from a population of 630,000 to 255 million per gm. of soil. At the end of 98 days, the bacterium grew as well in the presence of the antagonist as it grew alone (Table 1, Sec. I).

TABLE 1.—*The production of chloromycetin and the competitive interaction of Streptomyces venezuelae and Bacillus subtilis in soil*

Treatment,[a] and day of sampling	Number of cells per gm. × 10^5		chloromycetin extracted (μgm./gm.)	pH
	B. subtilis	*S. venezuelae*		
I. *Both organisms introduced simultaneously*				
A. 0	0.63	.....	.....	5.90
7	157.0	.....	.....	5.70
20	275.0	.....	.....	5.15
36	264.0	.....	.....	5.53
98	220.0	.....	.....	5.65
B. 0	.....	2.0	.....	5.90
7	.....	23.3	0.0	5.57
20	.....	28.5	0.0	5.10
36	.....	18.2	0.58	5.45
93	.....	168.0	0.50	5.75
C. 0	0.63	2.0	.....	5.90
7	95.0	38.8	0.0	5.75
20	131.0	13.8	0.0	5.25
36	169.0	710.0	0.0	5.68
98	255.0	925.0	0.50	5.75
II. *Bacterium introduced 14 days before the actinomycete*				
A. 0	255.0	.....	.....	5.70
7	360.0	.....	.....	5.20
20	257.0	.....	.....	5.65
65	280.0	.....	.....	5.70
100	225.0	.....	.....	5.65
B. 0	.....	0.39	.....	5.90
7	.....	35.5	0.0	5.05
20	.....	48.2	1.12	5.60
65	.....	62.5	0.50	5.63
100	.....	945.0	0.82	5.85
C. 0	255.0	0.39	.....	5.70
7	380.0	53.0	0.0	5.25
20	299.0	209.0	0.0	5.70
65	255.0	90.0	0.0	5.65
100	280.0	275.0	0.84	5.75
III. *Actinomycete introduced 14 days before the bacterium*				
A. 0	0.18	.....	.....	5.90
7	160.0	.....	.....	5.20
20	173.0	.....	.....	5.68
65	230.0	.....	.....	5.58
100	205.0	.....	.....	5.80
B. 0	.....	38.7	0.0	5.45
7	.....	12.0	0.0	5.05
20	.....	37.0	0.0	5.63
65	.....	30.0	0.50	5.45
100	.....	1230.0	0.79	5.78
C. 0	0.18	38.7	0.0	5.45
7	3.8	9.0	0.0	5.03
20	10.4	36.8	0.0	5.65
65	155.0	80.0	0.0	5.63
100	155.0	1230.0	0.60	5.83

[a] A, *B. subtilis* control; B. *S. venezuelae* control; C, *B. subtilis* plus *S. venezuelae*.

When introduced into soil before the antagonist, *B. subtilis* grew as well in the mixed cultures as it did in the control (Table 1, Sec. II). In both cases, a high point of growth was reached in 7 days after introduction of the actinomycete, the population of the bacterium reaching 36 to 38 million cells. Thereafter the population leveled off to between 22.5 and 28 million cells at the end of 100 days.

The bacterium grew only slowly when forced to compete with an already established population of *S. venezuelae* (Table 1, Sec. III), but no real inhibition took place. After 14 weeks' growth, the bacterium reached a population of 15 million cells in the mixed culture as compared with 20 million in the control. Statistical analysis indicated that this difference of 5 million cells was not significant. The rate of growth of the bacterium was most rapid during the first 7 days of incubation; the population increased twenty-fold. Thereafter, the weekly increase was approximately threefold until, after 65 days, growth leveled off at about 15.5 million cells. In all three groups, the maximum growth of *B. subtilis* in the controls was achieved after approximately a 3-week incubation period.

In all three experiments, the growth of *S. venezuelae* in the controls was characterized by a slow, fluctuating increase during approximately the first nine weeks of incubation, then a sharp increase. This increase occurred much earlier in those experiments in which the bacterium was introduced either simultaneously with or 14 days before the antagonist (Table 1, Sec. I, II), and seems to be correlated with a large population of *B. subtilis*. The possibility that the bacterium decomposes some constituent of the soil which can be attacked only very slowly by *S. venezuelae*, thereby providing the actinomycete with nutrients which stimulate its growth, is supported by the fact that the stimulation of the actinomycete was not pronounced in those mixed cultures where *B. subtilis* was slowly establishing itself against an already established population of the antagonist (Table 1, Sec. III). In any case, despite its ability to produce an active antibiotic in the soil, *S. venezuelae* was not as good a competitor as *S. griseus* (12).

The effect of fertilizers on chloromycetin production in soil.—Because of the extremely small amounts of chloromycetin produced by *S. venezuelae* in soil cultures, it seemed likely that nutritional factors were limiting and that the production could be increased by appropriate fertilizers. This hypothesis is not unrealistic since, through agronomic practices and natural processes, animal and plant residues are constantly incorporated into the soil. The production obtained with the use of corn stover, oat straw, soybean straw, and green alfalfa hay was compared with that obtained with two laboratory materials, tryptone and soluble starch. Since chloromycetin is a nitrogen-bearing compound, it seemed pertinent also to study the effect of narrowing the C/N ratio of several of the addenda to 10/1 with $(NH_4)_2SO_4$.

The soils were amended with the carbon source to 1 per cent by weight, sterilized, and inoculated with 1 ml. of a spore suspension of *S. venezuelae*. The production of chloromycetin was determined at the end of 18 and 31 days of incubation.

By far the largest production of chloromycetin was

given by tryptone—about 25 μgm. and 27.8 μgm. per gm. of soil at the end of 18 and 31 days respectively. Of the agricultural materials alfalfa was the most productive, but only to the extent of 1.4 μgm. Corn stover and soybean straw yielded trace amounts; oat straw and starch gave no detectable production of chloromycetin. The C/N ratio did not materially affect production since adjustment of the ratio with $(NH_4)_2SO_4$ in no instance increased the amount of detectable chloromycetin. The population density was not a decisive factor, since a heavier mass of aerial mycelia was observed in the starch flasks than in the alfalfa-treated soils; yet starch did not yield detectable antibiotic. It is probable that specific precursors in easily available form are most important to chloromycetin production. The unamended soil and most of the plant residues were not favorable substrates.

Identification of chloromycetin in soil.—That the inhibitory agent produced by *S. venezuelae* in soil is chloromycetin was confirmed by the following:

1. Chemical evidence. It has already been pointed out that the presence of an aromatic nitro group in the chloromycetin molecule is a unique property. Active soil extracts when reduced with $SnCl_2$ underwent a diazotization reaction indicating the presence of an aromatic group. Similar compounds were not found in control soils. An aromatic amine is excluded since the reaction was not obtained with unreduced extract.

2. Correlation between biological and chemical assays. Where both assays were used, the chloromycetin values of active soil extracts as determined by the bioassays and the colorimetric procedure agreed closely.

3. The R_F values of the soil extract. The calculation of R_F values by paper-strip chromatographic analysis is widely used as a means of identification of antibiotic substances. The R_F is the ratio of the distance which a chemical moves on a filter paper, when one edge of the filter paper is placed in a suitable solvent, and the distance which the solvent moves in the same length of time. Under standard conditions the R_F value is constant and specific for each chemical in a particular solvent system. Thus, if the R_F value of an unknown compound is compared to that of a known material, one criterion of identity can be established. The specific method used for the identification of chloromycetin in soil extracts is an improved technique for antibiotics developed by Miller (7). R_F values were determined on the extract from the tryptone-amended soil only, since the test sensitivity requires about 100 μgm. per ml. of active material. Three solutions were run simultaneously on the strips: 1) a chloromycetin standard of 100 μgm. per ml., 2) the soil extract unknown, and 3) a mixture of equal parts of standard and soil extract. The chromatograms were developed by the upflow method from two solvent systems at 26° C., water saturated with n-butanol, and n-butanol saturated with water.[4] The movement of the antibiotic on the paper strip was determined by the position of the zone of inhibition when the filter paper was placed on a large *B. subtilis* assay plate.

TABLE 2.—*Results of addition of chloromycetin to an established population of Bacillus subtilis[a] in soil*

Chloromycetin added (μgm. per gm.), and cultural treatment	Days	Chloromycetin recovered, μgm. per gm.	Viable cells, × 10^6
0—viable cells	0	.....	30
	0	.....	45[b]
10—heat-killed cells	0	7.0	0
	8	7.1	0
Viable cells	0	7.0	30
	8	7.0	36
15—heat-killed cells	0	11.5	0
	8	11.5	0
Viable cells	0	11.5	30
	8	10.6	32

[a] *B. subtilis* was grown for 18 days in the soil before chloromycetin was added. The soils were then incubated an additional 8 days.

[b] The population difference was statistically significant at the 5 per cent level.

The R_F values obtained with the butanol-saturated water system were as follows: standard, 0.70; soil extract, 0.71; mixture, 0.70. Those obtained with the water-saturated butanol were: standard, 0.88; soil extract, 0.88; mixture, 0.88. These values clearly show the identity of the active principle in the extract with chloromycetin.

Effect of B. subtilis on chloromycetin in soil.—The long growth period necessary for the appearance of chloromycetin when *S. venezuelae* is grown in soil containing *B. subtilis* cannot be due to the breakdown of the antibiotic by the bacterium (Table 2). No appreciable difference was found in the recovery values for chloromycetin obtained at the beginning and end of the 8-day incubation period in the presence of large numbers of bacteria. At both the 10- and 15-μgm. levels, the bacterium exerted no effect on the antibiotic. On the other hand, 15 μgm. per gm. significantly reduced the growth of the bacterium. Neither concentration was lethal since *B. subtilis* was able to multiply in those soils containing 10 μgm. and to hold its own in the presence of 15 μgm. of chloromycetin per gm.

Throughout the experiment, *B. subtilis* remained sensitive to chloromycetin as indicated by a complete lack of growth at 1:100 dilutions on chloromycetin-amended agar. Therefore, the survival of the bacterium in the presence of the antibiotic in soil cannot be attributed to the evolution of a chloromycetin-resistant population. The data indicate that the only effect of the concentrations of antibiotic used was to retard rather than inhibit the bacterium. This may

[4] Paper-strip chromatograms were made by Dr. Richard Miller of the Division of Biochemistry, University of Illinois.

TABLE 3.—*Response of the soil microflora to addition of 50 μgm. chloromycetin per gm. of soil*

Treatment	Days	Number of viable cells per gm. of soil							
		Bacteria × 10^5		Actinomycetes × 10^5		Fungi × 10^4		Total × 10^5	
		G[a]	CR[a]	G	CR	G	CR	G	CR
Soil control	0	303.7	24.5	66.8	7.9	19.3	16.3	372.4	34.0
	7	313.3	30.0	83.3	15.0	17.0	34.8	398.4	48.5
	14	203.3	15.2	80.0	12.5	20.3	15.3	285.3	29.2
Soil plus chloromycetin	0	303.7	24.5	66.8	7.9	19.3	16.3	372.4	34.0
	7	563.3	22.0	118.3	17.0	33.4	46.0	684.9	43.6
	14	271.7	29.7	98.3	13.5	17.7	17.3	371.8	44.9

[a] G = general numbers of organisms; CR = number of colonies developing on agar amended with 10 μgm. of chloromycetin per ml.

explain why *B. subtilis* was not inhibited in association with *S. venezuelae*, even though it is possible that small amounts of chloromycetin had been synthesized and had diffused into the soil. The results, however, do not explain the lag in appearance of detectable chloromycetin in the mixed cultures, unless the bacterium was able to attack minute amounts of the antibiotic gradually elaborated into the soil medium. It is also possible that some activity of *B. subtilis* affected the metabolism of *S. venezuelae* in a way antagonistic to the production of chloromycetin.

The effect on chloromycetin of the microflora of normal soil.—That chloromycetin can be produced in soil by *S. venezuelae* has been demonstrated. The actinomycete has a cosmopolitan existence; it has been isolated from soils from Japan and Venezuela and from Indiana and Illinois in the United States. Nevertheless, it is questionable that chloromycetin could accumulate in a natural soil containing a large number of microorganisms. Pure cultures of a number of bacteria can, in the laboratory, inactivate or destroy chloromycetin either by hydrolyzing the molecule or by reducing the nitro group (14, 15). Accordingly, at least some members of such a physiologically diverse group as the soil microflora should be able to act on the antibiotic. If true, this information on the effect of the indigenous soil population would throw some light on the probable fate of any chloromycetin produced in a natural soil under the impact of intense biological activity.

From figure 2 it is apparent that a large proportion of the 50 μgm. of chloromycetin added to a gram of nonsterile soil disappeared. Approximately 39 μgm. could be recovered at the start of the experiment, but only about 5 μgm. could be recovered at the end of 14 days—a loss of 85 per cent. After an initial lag period of one day, the disappearance of active chloromycetin was rapid, followed by a distinct leveling off after the seventh day. Between the first and third days there was 43 per cent loss of activity, 38 per cent decrease during the next four days, and 50 per cent decrease the following week. The recovery of the antibiotic from sterile soil was relatively constant, the values fluctuating around ± 2 μgm. A comparison of the two assay procedures on extracts from sterile soil reveals a random error not exceeding 4 μgm. per gm. The loss in chloromycetin in the nonsterile soil clearly was due to biological activity.

The tests were inconclusive with regard to detection of intermediate breakdown products of chloromycetin. Tests for aryl amines were completely negative throughout the course of the experiment. The data do show a small but consistent difference between the chemical and biological assays of the extracts from the nonsterile soil. The recovery values given by the colorimetric determination were always higher than those by the biological assay. This difference could be attributed to experimental error, though it also might be a measure of a small quantity of aromatic nitro residues from chloromycetin breakdown. In any case, there appears to have been a decided uptake of the antibiotic or its degradation products by the soil microorganisms.

A number of conclusions may be drawn from the data in table 3:

1. The effect of the addition of chloromycetin to nonsterile soil was most pronounced on the bacterial population, which almost doubled in the treated soils in 7 days, whereas only a slight increase was recorded for the numbers of bacteria in the soil controls during the same period.

2. The population changes of the actinomycetes in both treated and untreated soils were of the same magnitude, indicating no particular response to the addition of chloromycetin to the soils. The fungus population remained relatively stable.

3. The addition of chloromycetin to the soil did not appear to select out a population resistant to the antibiotic. About the same percentage of the total number of microorganisms appearing on the agar plates was resistant to 10 μgm. per ml. at the beginning and end of the 14-day incubation period. Although the bacteria were stimulated by the addition of the antibiotic to the soil, they remained primarily chloromycetin-sensitive on laboratory media. As would be expected, sensitivity to the antibiotic was most pronounced among the bacteria and actinomycetes. The fungi appeared to be relatively insensitive.

DISCUSSION OF RESULTS.—Activity and adsorption studies on the neutral antibiotic, chloromycetin, reveal that, unlike the basic material streptomycin, it is potentially active if produced in the soil. This activity is associated with the compound's ability to exist free in the soil or in weakly bound form, as is indicated by the relative ease with which it can be eluted from the soil by water or organic solvents.

The actual level of activity is considerably masked by the soil. Sixty times as much chloromycetin was required to inhibit *B. subtilis* in soil as was required in broth. Therefore it should be emphasized that the inhibitory concentrations of an antibiotic in laboratory media are inadequate criteria for its effect in the soil.

That *S. venezuelae* can utilize the soil substrate as a medium for the production of chloromycetin is very important, for it indicates that in this case antibiotic production is a normal physiological mechanism and not an aberrant phenomenon appearing only under laboratory conditions of culture. The large amount produced with tryptone amendment should not be regarded as any index of production in field soil; tryptone is a specially prepared nutrient and hardly representative of the materials which ordinarily find their way into the soil. A much more realistic index was the production under alfalfa-hay amendment.

While the growth of the actinomycete on unamended soil gave rise to a small quantity of detectable chloromycetin, production was not increased with oat straw or starch. This result indicates that a preferential feeding not conducive to the production of the antibiotic took place in those amended soils. In other words, the oat straw and starch constituted the main substrates for the actinomycete's metabolic activities and did not provide the specific precursors for the synthesis of chloromycetin. On the other hand, a very small amount of such precursor must have been present in the soil itself.

The capacity to produce an active antibiotic is not necessarily correlated with an organism's competitive ability in soil. *S. venezuelae* was not able to suppress the growth of *B. subtilis* in mixed soil cultures, whereas *S. griseus*, although incapable of producing an active antibiotic in the soil, was highly antagonistic to the bacillus (13).

The amounts of chloromycetin added to the soil (50 μgm. per gm.) in the soil stability studies are probably much greater than could actually be produced under field conditions. Nevertheless, the soil population showed a marked ability to metabolize the compound and quickly neutralize the effects of its production in soil.

It can be concluded that chloromycetin is potentially able to play a significant role in biologic equilibria in the soil in terms of its adsorption and activity status. However, the results actually obtained, when translated into field conditions, indicate that the production and accumulation of chloromycetin in soil might be, at best, only negligible.

SUMMARY

Chloromycetin can be produced by *S. venezuelae* when grown in unamended soil.

The production of chloromycetin could be greatly increased by the addition of tryptone and only slightly by the addition of green alfalfa hay. Oat straw and starch were ineffective.

S. venezuelae was unable to antagonize the growth of *B. subtilis* in unamended soil.

Chloromycetin was active in the soil although the activity level was below that in broth.

Chloromycetin added to a nonsterile soil was rapidly degraded by members of the soil population.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

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FREQUENCY OF BACTERIAL BLIGHT-RESISTANT PLANTS IN TWENTY COTTON VARIETIES[1]

L. A. Brinkerhoff, John M. Green, Richard Hunter, and George Fink[2]

Bacterial blight is one of the major diseases of cotton in Oklahoma and adjacent cotton-producing areas. Chemical seed treatments reduce primary infection but do not prevent epidemics of the disease. The development of resistant varieties appears to be the most feasible control measure.

Commercial varieties of American upland cotton (*Gossypium hirsutum* L.) differ in their susceptibility to bacterial blight, but none are highly resistant (6, 7, 9). Complete resistance does exist, however, in some of the Old-World cottons cultivated in Asia (6, 7).

The first resistant American upland cotton, discovered in Africa about 1934 (5), was a nonproductive type and was designated Nye's Uganda B_{31}. By backcrossing and rigid selection in artificially inoculated breeding plots, Knight (2, 3, 4) and Knight and Clouston (5) have transferred resistance from Uganda B_{31} and other resistant types to the highly susceptible Egyptian varieties (*G. barbadense* L.). Knight and associates (6, 7) have made extensive surveys of both Old-World and New-World types of cotton. Resistance was found in many of the Old-World cottons, but rarely in American upland varieties except those previously grown in Asia or Africa. Five major genes for blight resistance have been reported by Knight and associates.

In the United States, Simpson selected a blight-resistant strain of cotton from Stoneville 2A in 1939. It was designated Stoneville 20 and described by Simpson and Weindling (9) in 1946. Stoneville 20 has not been grown on a commercial basis, but has been widely used in crosses with commercial upland varieties. Blank (1) reported in 1949 that resistance in Stoneville 20 appeared to be controlled by a single recessive gene.

During the past 5 years bacterial blight-resistant plants have been found by other investigators in the United States. Moosberg (see 8) has developed a resistant strain from three plants selected in naturally infected Stoneville 2B. Hooton (see 8) isolated resistant plants in 1948 from Floyd Mebane and Buckellew Mebane, and Blank (see 8) found resistant plants in Hurly Rowden in 1949. Also in 1949 the senior author found resistant plants in an artificially inoculated planting of Mebane 6801 and among naturally infected plants of an unknown variety. In most instances only an occasional resistant plant has been found in relatively large populations in American upland varieties.

The present study comprised a survey of 18 commercial varieties and breeders' strains of upland cotton (*G. hirsutum* L.) and two varieties of American-Egyptian cotton (*G. barbadense* L.). Relatively large populations (13,000 to 25,000 plants) were surveyed, as contrasted to small populations (23 or 46 hills) observed by Knight and Hutchinson (7) and single-progeny rows by Knight and Clouston (6).

MATERIALS AND METHODS. — Approximately 32,000 seeds of each of 20 varieties were planted in a randomized block design with eight replicates. The varietal designation and the source of each are in table 2. Plots consisted of four rows, each 100 ft. long. Approximately 10 seeds were planted per foot of row space. Thinning was delayed until after the survey was completed. The test was planted on May 17 and 18, 1950, and inoculated during the third week of June.

The method of inoculation was a modification of techniques developed by Knight and Clouston (5), Simpson and Weindling (9), and Weindling (10). Six different isolates of the blight bacterium, *Xanthomonas malvacearum* (E. F. S.) Dowson, were grown in a liquid medium and a composite culture used at the rate of 1 pt. to 100 gal. water.[3] Two men, each using a single-nozzle spray gun of a power sprayer operated at about 400 psi, applied 100 gal. of diluted inoculum to approximately 2,000 ft. of row space. The spray stream was adjusted to produce visible water-soaking of the leaves without serious mechanical injury. Water-soaking on the underside of the leaves was obtained after about 7 a.m. and as long as the plants remained turgid.

Blight symptoms were evident 5–7 days after inoculation and grading was started 14–16 days later, or 3–4 weeks after inoculation. Unseasonably cool weather probably retarded symptom development, but a very severe epidemic developed.

Plants were counted and examined rapidly for leaf infection. Those that were not infected or had small atypical lesions were tagged and evaluated later in the season. Abundant secondary infection favored by driving rains obviated the need for further inoculation. Tagged plants which had previously been escapes were discarded and the remaining plants graded according to leaf reaction.

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[2]Respectively, Associate Pathologist, Agronomist, and former research assistants. L. A. Brinkerhoff and John M. Green are employed jointly by the Oklahoma Agricultural Experiment Station and the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S. Department of Agriculture.

The writers wish to acknowledge the assistance of Stuart Evans and John E. Chilton in developing the inoculation technique.

[3]The liquid culture medium was made up as follows: peptone, 5 gm.; beef extract, 3 gm.; juice from 300 gm. of boiled potatoes and 50 gm. of boiled carrots; water to make 1,000 ml. The pH was adjusted to 6.8–7.6.

A numerical scale somewhat similar to one suggested by Weindling (10) was first used, 4 to 5 weeks after inoculation. This scale included grades 0, 1, 2, 3, and 4 and was based on the presence or absence, relative size, and appearance of individual lesions on the leaf blade. Plants with no discernible lesions on the leaves except for occasional dry brown-to-black lesions along the veins were classed as grade 0. Grade 1 was characterized by dry, very small round lesions that other investigators (5, 9, 10) have considered as representative of a highly resistant reaction. Grade 2 consisted of a tolerant reaction identified by typical green angular spots 1–2 mm. in diameter, which sometimes coalesced but tended to dry rapidly; vein blighting was limited, mainly brown. Lesions of grade 3 were somewhat larger than those of grade 2, and tended to coalesce more frequently; vein blighting was more abundant and considerably more water-soaked. Grade 4 represented a fully susceptible leaf-blade reaction; large green angular spots dried slowly, and bacterial exudate was abundant. Vein blighting was always water-soaked, but varied in amount with different varieties.

Six to 8 weeks after inoculation it was necessary to add two additional grades because all of the plants that originally graded 0 and many of those that graded 1 and 2 developed a few relatively large water-soaked lesions. Thus eventually the following seven grades were employed: 0.2, 1, 1.2, 2, 2.3, 3, and 4. The first three represent resistant reactions as described above, the second three represent tolerant reactions, and the last grade a fully susceptible reaction. Stoneville 20, which was used as the resistant standard, originally graded 0 but later developed a few scattered lesions that would have placed it in grade 0.2. A summary of the leaf symptoms appears in table 1. Five of the seven grades used in scoring blight resistance are illustrated in figure 1.

TABLE 1.—*Summary of leaf grades used in surveying cotton varieties for resistance to bacterial blight*

Grade	Leaf symptoms	
	3–4 weeks after inoculation	6–8 weeks after inoculation
0.2	Occasional dry brown-black lesions along veins.	Earlier symptoms plus a few scattered lesions on lamina characteristic of grade 2.
1	Dry, very small round lesions on lamina.	Same as earlier symptoms.
1.2	Same as grade 1.	Earlier symptoms plus scattered lesions characteristic of grade 2.
2	Angular spots 1–2 mm. in diameter, drying rapidly.	Same as earlier symptoms.
2.3	Same as grade 2.	Earlier symptoms plus larger spots characteristic of grade 3.
3	Larger spots than in grade 2, coalescing more frequently.	Same as earlier symptoms.
4	Large green angular spots.	Same as earlier symptoms.

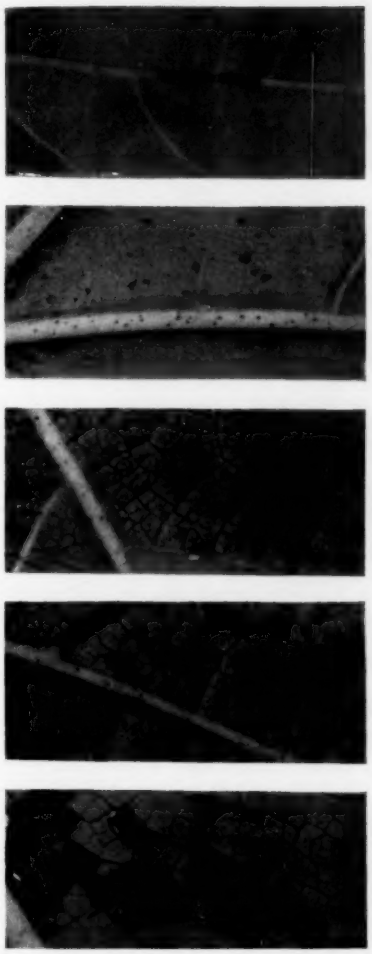

FIG. 1. Five of seven leaf grades used in scoring bacterial blight resistance. Top to bottom: grades 0.2, 1, 2, 3, and 4.

RESULTS.—Results of the survey are in table 2. Of the 20 varieties included, one yielded one resistant plant, nine yielded resistant and tolerant plants, and three yielded tolerant plants only. Five upland varieties, including one Rowden and four Açala strains, and two American-Egyptian strains failed to produce any resistant or tolerant plants. There was a wide range in frequency of plants having resistance or tolerance, with observed values from 1/24,597 for Stoneville 2B to 1/243 for Stormproof No. 1. The results are possibly subject to error resulting from resistant or tolerant plants being overlooked.

DISCUSSION.—Resistant plants were found chiefly in varieties that originated in the Texas-Oklahoma area. The only exceptions are the varieties Half and Half and Hi-Bred from Georgia. However, seed of Half and Half had been increased in the Texas-Oklahoma area. Only tolerant plants were found in the two varieties originating in Mississippi and in one of the two varieties from Arkansas; the other variety from Arkansas

TABLE 2.—*Frequency of resistant and tolerant plants in 20 cotton varieties examined for blight resistance following field inoculation*

Variety	Origin	Number of plants			Frequency[a]
		Examined	Resistant	Tolerant	
American upland					
CA 122	Texas	20,710	39	11	1/414
Stormproof No. 1	Texas	23,346	25	71	1/243
Mebane 6801	Oklahoma	24,675	15	4	1/1,299
Northern Star	Texas	24,414	13	2	1/1,628
CA 119	Texas	22,686	4	8	1/1,891
Lankart 57	Texas	14,221	3	1	1/3,555
Lockett 140	Texas	24,970	3	2	1/4,994
Half and Half	Georgia and Texas	20,057	2	2	1/5,014
Hi-Bred	Georgia	14,656	1	0	1/14,656
Stoneville 62–1	Oklahoma	20,738	1	17	1/1,152
Deltapine 15	Mississippi	25,892	0	17	1/1,523
Stoneville 2B	Mississippi	24,597	0	1	1/24,597
Arkot No. 1	Arkansas	22,385	0	2	1/11,193
Dortch No. 1	Arkansas	21,189	0	0	0/21,189
CR–1 (Acala 892)	Oklahoma	13,521	0	0	0/13,521
Acala 44	Arizona	25,796	0	0	0/25,796
Acala Santan	Arizona	23,756	0	0	0/23,756
Acala 4–42	California	22,211	0	0	0/22,211
American–Egyptian					
Pima 32	Arizona	21,415	0	0	0/21,415
Amsak	Arizona	17,946	0	0	0/17,946

[a]Includes resistant and tolerant plants.

had neither resistant nor tolerant plants. The total number of tolerant plants probably should have been somewhat greater, especially in such varieties as Deltapine 15, CA 119, and Stormproof No. 1. Many plants in these varieties approached grade 3 in tolerance but were discarded.

Although the four strains of Acala differed widely in their place of origin and development, neither resistant nor tolerant plants were found. Among these strains was CR-1 (Acala 892), which had been developed in Oklahoma by about the same method as employed for Mebane 6801 (selection in open pollinated breeding blocks). The other three Acalas were developed in Arizona and California; Acala Santan by selection in an isolated open-pollinated breeding block, and Acala 44 and Acala 4-42 by inbreeding.

It was not possible to evaluate the plants fully from an agronomic aspect because of severe boll-weevil damage. A majority of the resistant plants in Mebane 6801 and Lockett 140 were dwarfed, as were a few plants in CA 122. This characteristic was encountered in resistant Mebane 6801 plants isolated in 1949. It has not yet been determined whether this dwarfing is associated with the gene B_1, described by Knight and Clouston (5). The majority of both resistant and tolerant plants in other varieties appeared normal as to plant type and boll characteristics.

SUMMARY

Relatively large populations of 20 varieties of cotton from the United States were artificially inoculated with *Xanthomonas malvacearum* and later examined for resistant and tolerant plants.

Resistant plants were found in 10 of the 20 varieties surveyed; three additional varieties contained tolerant plants. The number of resistant or resistant and tolerant plants found in any one variety varied from one to 96. Resistant plants were found in greater numbers in varieties originating in Texas and Oklahoma.

OKLAHOMA AGRICULTURAL EXPERIMENT STATION
AND
U. S. DEPARTMENT OF AGRICULTURE
STILLWATER, OKLAHOMA

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BEAN VIRUS 2 (YELLOW) ON GREAT NORTHERN BEAN IN MONTANA [1]

M. M. Afanasiev and H. E. Morris

Some varieties and strains of Great Northern beans, University of Idaho (U. I.) 15, 81, and 123 and Montana 1, are highly resistant to bean virus 1. Nevertheless, when these strains are grown in Montana, another virosis frequently occurs on individual plants near the edges of commercial fields, along alleys or roads, and on the banks of irrigation ditches where numerous wild and escaped legumes grow. Since many acres of Great Northern beans are raised for certification, it becomes imperative to investigate the virosis and to identify the virus and study its properties, its host range, and the possibility of its transmission through seed.

FIELD SYMPTOMS.—Symptoms of the virosis on young trifoliate leaves of U.I. 15 bean plants first appear as slight, irregular mottling, and the leaflets droop somewhat at the pulvini. Small chlorotic spots often develop, enlarge, and coalesce; the leaves become crinkled, misshapen, and slightly chlorotic. Chlorotic mottling may follow the leaf veins, but more often it has no relation to them. Some veinclearing is on all leaves. Older leaves become rugose and curled, but no cupping has been observed. Internodes are short, plants are stunted or dwarfed and often spindly, maturity is delayed, and yield is poor. Some of these symptoms resemble those caused by yellow bean mosaic or bean virus 2 (5,7), but absence of pronounced chlorosis precluded an assumption that bean virus 2 was responsible for this virosis.

MATERIALS AND METHODS.—Most of the diseased bean plants and legumes were collected either at the Huntley Branch Station, Huntley, Montana, or in adjacent fields. They were immediately transported to Bozeman in an iced refrigerator and used as a source of inoculum within 24 hr. after collection.

Plants tested as hosts were Great Northern U.I. 15, U.I. 59, Montana 1, Pinto, Red Kidney, and Ideal Market bean, unless otherwise noted; broad bean (*Vicia faba* L.), white sweet clover (*Melilotus alba* Desr.), yellow sweet clover (*M. officinalis* (L.) Lamb.), alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), white and Ladino clover (*T. repens* L.), pea (Perfection, *Pisum sativum* L.), soybean (*Soja max* (L.) Piper), and a cultivated form of tulip, iris, and gladiolus.

Plants were inoculated by the method recommended by Rawlins and Tompkins (6) then lightly sprinkled with water and kept overnight in a moist chamber. Bean plants were in the early stage of the first trifoliate leaf. In all experiments several noninoculated plants were used as checks. The greenhouse tests were at Bozeman, Montana, and the field trials at the Huntley Branch Station.

SEED TRANSMISSION OF THE VIRUS.—Field observations indicate that the virus is not transmitted through the seeds, since no increase in the disease was observed when seeds from infected bean plants were planted. Continuous occurrence of this disease in the border rows indicated that it is probably introduced each season from without.

No transmission of the virus occurred when 930 bean plants were grown in the greenhouse from seed collected from diseased field plants. Growth of some plants was rather weak, leaves were elongated and slightly crinkled, with some rugosity and chlorosis. Some leaves also had small yellowish spots. All these abnormalities tended to disappear with age.

During 1942 and 1943, when 320 plants were grown in the field from seeds collected from field-grown diseased bean plants, less than 2 per cent showed the disease. Since plants in adjacent rows grown from seed of healthy plants had a higher percentage of disease, probably all the disease was due to current-year infections, and was not transmitted through the seed.

Seeds from the most abnormal plants grown in the greenhouse during the winter of 1941-1942 were planted in the greenhouse to determine whether some of these abnormalities will transmit through the seeds. Most of the 150 plants grown were normal; the abnormalities that appeared in a few plants probably were due to unfavorable growing conditions during the winter months.

Seeds of the plants that had been inoculated and that had positive symptoms of disease during 1944 and 1945 were planted in the greenhouse. No transmission of the disease occurred.

BEANS AS A SOURCE OF INOCULUM.—*Field Beans*: During the summer and fall of 1944, 1945, and 1946, six, nine, and four transmission experiments were made in the respective years. Healthy plants of several bean varieties and other leguminous plants were inoculated (Table 1). All varieties of beans, white and yellow sweet clovers, and broad bean became infected, but red, alsike, and white clovers and alfalfa did not. Much greater percentages of infection were obtained in 1945 and 1946 than in 1944.

Symptoms of this virosis on U.I. 15 and U.I. 59 in the greenhouse were identical to those described for U.I. 15 in the field, except that the greenhouse plants showed more yellowing and were more spindly.

Symptoms on Montana 1 were usually mild, only a faint mottling and some crinkling, but practically no rugosity, appearing on the upper leaves of the slightly dwarfed plants. Only one plant of Montana 1 had symptoms similar to those of U.I. 15.

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TABLE 1.—*Infection of bean varieties and other legumes by virus inoculum secured from various field plants*

Inoculum source[a]	Number of plants infected / Number inoculated					
	Bean variety	1944	1945	1946	Other legumes[b]	1945	1946
Bean, Great Northern	Red Kidney	27/32	13/26	.....	Sweet clover:		
	Montana 1	7/38	19/26	.....	White	11/12	5/12
	Pinto	.....	20/26	.....	Yellow	6/12	8/12
	U.I. 15	9/38	18/26	13/25	Broad bean	.....	2/11
	U.I. 59	.....	20/26	6/25			
	Ideal Market	10/32	.....	.....			
Bean, Red Kidney	Red Kidney	.....	2/8	.....			
	Montana 1	.....	1/8	.....			
	Pinto	.....	6/8	.....			
	U.I. 15	.....	7/8	.....			
	U.I. 59	.....	6/8	.....			
Sweet clover, white	Red Kidney	0/4	0/9	.....	Sweet clover:		
	Montana 1	.....	0/9	.....	White		8/12
	Pinto	.....	0/9	.....	Yellow		7/12
	U.I. 15	0/4	0/9	1/20	Broad bean		0/6
	U.I. 59	.....	1/9	3/20			
Sweet clover, yellow	Red Kidney	.....	3/9	.....	Sweet clover:		
	Montana 1	.....	3/9	.....	White		4/5
	Pinto	.....	1/9	.....	Yellow		5/6
	U.I. 15	.....	0/9	2/10	Broad bean		0/3
	U.I. 59	.....	4/9	1/10			
Alfalfa	Red Kidney	0/14	0/5	.....	Sweet clover:		
	Montana 1	2/14[c]	0/5	.....	White		0/3
	Pinto	.....	0/5	.....	Yellow		0/3
	U.I. 15	2/14[c]	0/5	0/20	Broad bean		0/3
	U.I. 59	.....	1/5[c]	0/20			
	Ideal Market	0/14	.....	.....			

[a] In 1944 inoculum from red clover and from alsike clover failed to infect Red Kidney, Montana 1, U.I. 15, and Ideal Market beans.
[b] None of the inoculated plants of red, alsike, Ladino, and white clovers and of Ranger alfalfa became infected.
[c] Symptoms somewhat similar to, but not typical of, virosis.

Symptoms on Pinto closely resembled those on U.I. 15. Young inoculated Pinto plants often remained stunted for about a month, then produced a spindly growth with typical virosis symptoms.

On Red Kidney beans mottling, crinkling, rugosity, and veinclearing appeared. Older leaves were rugose, spindly, and yellow, with small islands of green tissue throughout the leaf blade. In general the symptoms on Red Kidney were somewhat milder than those on either Great Northern or Pinto.

Two of the inoculated plants of Ideal Market had symptoms identical to those of Red Kidney, but several others developed entirely different symptoms. Terminal shoots stopped growing and the plants became dwarfed and stiff. The leaves rolled and became chlorotic and blotchy with brownish-red vein discoloration, and the plants died even though the roots were in good condition. This behavior was somewhat similar to that of Pinto, except that Pinto finally produced new growth. Pierce (5) reported similar effects of bean virus 2 on Mexican tree beans.

Numerous Red Kidney beans grown at the Huntley Branch Station had symptoms similar to those of the inoculated plants. Plants of several bean varieties were inoculated with the juice of field-grown diseased Red Kidney plants. A high degree of transmission was obtained (Table 1) and the symptoms of the inoculated plants were similar to those of the virosis studied.

Both white and yellow sweet clover plants were susceptible and had light-green blotches interspersed with dark-green areas similar to those observed in the field.

The symptom on broad bean was a slight chlorotic mottling of leaves.

Greenhouse beans: During 1944, 1945, and 1946 bean plants inoculated with the virus and which developed good symptoms of the disease in the greenhouse were used to inoculate eight series of healthy bean plants. Transmission was slight: Virus from Great Northern bean infected one of 14 plants of U.I. 15; one of five plants of U.I. 59; and none of 15 Red Kidney and three broad bean plants. Virus from Red Kidney infected one of 16 Red Kidney plants and none of four plants of U.I. 15, and virus from Ideal Market failed to infect either Red Kidney (four plants) or Ideal Market (four plants).

Lack of transmission may be because of the nature of the virus, or because of masking symptoms. The tests were made during late fall or in winter when neither temperature nor light conditions in the greenhouse were satisfactory for growing beans.

OTHER SOURCES OF INOCULUM.—Diseased plants of white and yellow sweet clover, alfalfa, red clover,

alsike clover, soybeans, pea (Perfection), and some liliaceous and iridaceous plants were used as inoculum.

White and yellow sweet clover.—Composite samples of field-grown white sweet clover with mosaic symptoms in the form of light-green blotches were collected in July or August and used as inoculum for one series in 1944, two in 1945, and four in 1946. Several varieties of beans and other legumes were inoculated (Table 1). No virus transmission was obtained in 1944, and only one inoculated bean plant had disease symptoms in 1945. Much better transmission was obtained in 1946; several bean plants and a high percentage of sweet clover had symptoms of the virosis studied.

Composite samples of field-grown yellow sweet clover with symptoms of mosaic similar to those of white sweet clover were collected in July or August and used as inoculum in four series of experiments in 1945 and 1946. Transmission of the virus to bean and to a high percentage of sweet clover was obtained, but none to common and Ranger alfalfa and broad bean.

Plants of white and yellow sweet clover, in which good symptoms developed after virus inoculations in the greenhouse, were used as inoculum in eight experiments with white and two with yellow clover to study the overwintering of the virus. The plants were first rested in a cold room for 2½ months; the old leaves and stems died during this period but new growth developed later in the greenhouse. The results with white sweet clover inoculum were: U.I. 15, 1/38[2]; U.I. 59, 0/38; white sweet clover, 1/3; yellow sweet clover, 1/3; broad bean, 4/6. Those with yellow sweet clover inoculum were: U.I. 15, 1/10; U.I. 59, 0/10; white sweet clover, 0/3; yellow sweet clover, 2/3; and broad bean, 1/3. Definite transmission was obtained, although the percentage was low. Thus the virus may overwinter in sweet clover plants and they may serve as a source of primary infection for beans.

In the spring of 1946, bean plants of U.I. 15 and U.I. 59 were inoculated with the juice of field-grown young sweet clover plants showing leaf blotching, but results were negative. It is possible that the concentration of the virus in the young growth was not sufficient to transmit the disease. Transmission from older plants was obtained in other experiments.

Alfalfa.—Composite samples of field-grown alfalfa leaves having a virosis in the form of mottling were used as inoculum in seven experiments during 1944 to 1946 (Table 1). In 1944 and 1945, five inoculated bean plants had symptoms that resembled the virosis somewhat but were not typical. Other inoculated plants had symptoms entirely different from those of the virosis under investigation. Tests in 1946 gave similar results. It is uncertain and rather questionable that alfalfa carries the disease in Montana.

Red clover.—Two composite samples of red clover leaves infected with a virosis in the form of mottling were collected in 1944 at Bozeman, Montana. Four varieties of beans (U.I. 15, Montana 1, Red Kidney, and Ideal Market) were inoculated with this material but no transmission occurred.

Alsike clover.—One composite sample of leaves of alsike clover infected with a virosis producing yellow mottling of leaves was collected at Bozeman, Montana. Four varieties of bean (U.I. 15, Montana 1, Red Kidney, and Ideal Market) were inoculated with this material but again no transmission occurred.

Tulip, iris, and gladiola.—McWhorter et al. (4) have shown that gladiolus plants, in addition to different leguminous plants, serve as a reservoir for yellow bean mosaic in Oregon. Since the virosis studied, according to preliminary information obtained, may be related to yellow bean mosaic, it was decided to test various plants of the lily family grown in Montana for the occurrence of the virus.

Tulips, irises, and gladiolus plants grown in Bozeman, having mosaiclike mottling, were used as inoculum in nine experiments during the spring of 1947; tulip in four, iris in three, and gladiolus in two. In each experiment five healthy bean plants of each of U.I. 15 and U.I. 59 were inoculated with the juices extracted from the liliaceous plants. The results of these tests were negative, which gives some indication that the virus studied in this investigation apparently is not related to one described above (4).

Reactions of pea and soybean plants.—Plants of Perfection pea and soybean were inoculated with juice of diseased Great Northern beans to determine whether the virosis under investigation is caused by the original or a new strain (2, 3, 4) of yellow bean mosaic. A considerable number of the soybean plants developed crinkled leaves with yellow mottle, but all peas remained free of disease.

Resistance of different selections of Great Northern beans.—For several years numerous lines of Great Northern beans resistant to bean virus 1 were tested in the field for resistance to the virosis under investigation. Only 14 lines were resistant; these, together with U.I. 123, were grown in the greenhouse during the summers of 1947, 1948, and 1949, and further tested by artificial inoculation with the virus from field-grown Great Northern beans. Only two selections, Montana 1 and 43-15, consistently showed a high degree of resistance in the greenhouse. In most cases these selections had no symptoms, or very mild ones which were difficult to detect.

Properties of the virus.—Virus from the juice of a diseased Great Northern bean plant was used for dilution, thermal inactivation, and aging studies. The test plants inoculated were the bean varieties U.I. 15, U.I. 59, Montana 1, Pinto, and Red Kidney.

The virus was transmitted in dilutions of 1:1, 1:10, 1:100, and 1:000, but higher dilutions (1:2000, 1:5000, 1:10,000, and 1:1,000,000) failed to produce virosis in the inoculated beans.

Heating at 60° C. for 10 min. inactivated the virus,

[2] Number of plants infected/number of plants inoculated.

and none of the inoculated bean varieties became infected.

The inoculum was infectious if used immediately after extraction, or after aging for 6 or for 18 hr. No transmission was obtained after the inoculum aged for 30 or for 42 hr.

DISCUSSION.—On the basis of information available, an attempt was made to identify the virus. Zaumeyer and Harter (8) proposed the following method for the identification of bean viruses. They state that it would be difficult, if not impossible, to differentiate bean virus 1 and 2 and bean mosaic virus 4 and 4A on the basis of symptomatology on certain varieties in the field, but that differentiation is possible on certain varieties grown under controlled conditions and by determining the properties of the viruses.

Bean virus 1 and bean mosaic virus 4A are transmitted through the seed and bean virus 2 and bean mosaic virus 4 are not. On this basis it is evident that the virus studied in this investigation is not related to either bean virus 1 or bean mosaic virus 4A.

Bean mosaic virus 4 can be separated from bean virus 2 by inoculating plants of Pinto bean on which bean mosaic virus 4 produces local lesions and bean virus 2 produces mottle symptoms on the trifoliate leaves. In the present investigation the virus studied produced general mottling on Pinto leaves. For this reason it is evident that this disease has no connection with bean mosaic virus 4.

The host differentiation, the properties, and the association of this virus with white and yellow sweet clovers indicates that this virus belongs in the group of bean virus 2 (yellow bean mosaic). The results of recent investigations indicate that bean virus 2 is composed of several strains (2, 3, 4) and also is closely related to bean virus 1 (1).

Zaumeyer and Wade (7), by inoculation tests, found that Great Northern Idaho No. 1, Robust, California White, and Red Kidney (Dark Mahogany Strain) beans are resistant and Perfection pea susceptible to the white sweet clover mosaic virus. Pierce (5), by inoculation tests, found that Great Northern Idaho No. 1 and Robust beans are susceptible, and Perfection pea resistant to yellow bean mosaic. Grogan and Walker (2) found that Wisconsin Perfection pea was resistant to typical bean virus 2 and the pod-distorting virus, but that soybean was susceptible to typical bean virus 2, and resistant to the pod-distorting virus. McWhorter and Boyle (3) found that varieties of the Perfection type of pea were particularly susceptible to a newly described strain of yellow bean mosaic.

The results of inoculation tests and property studies indicate that the virus occurring in Great Northern beans in Montana is identical, or closely related, to yellow bean mosaic described by Pierce (5).

Some resistance to this virosis was found in two strains of Great Northern beans grown in Montana.

SUMMARY

Bean plants infected with a virosis occurring on Great Northern bean, a variety resistant to bean virus 1, develop drooping of leaflets, chlorotic spotting, and later general mottling, distortion, and crinkling of leaves; they gradually become rugose, dwarfed, and spindly, grow slowly, bloom late, and yield poorly. The virus does not transmit through the seed.

Infection resulted when infectious plant juice was inoculated into Great Northern beans U.I. 15, U.I. 59, Montana 1, Red Kidney, Pinto, Ideal Market, broad bean, soybean, and white and yellow sweet clover.

Bean varieties became infected when inoculated with juice from field-grown diseased plants of white and yellow sweet clover and Red Kidney bean. The sweet clovers apparently serve as main reservoir of this virosis in nature.

In property studies, this virus inactivates in a dilution greater than 1:1000, also by heating for 10 min. at 60 C., and by aging for more than 18 hr.

Some resistance to this virosis was found in two strains of Great Northern beans (Montana 1 and 43-15) grown in Montana.

The host differentiation, the properties, and the association of this virosis with white and yellow sweet clovers indicates that this disease is caused by bean virus 2, as described by Pierce (5).

MONTANA AGRICULTURAL EXPERIMENT STATION
BOZEMAN, MONTANA

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EPIDEMIOLOGY AND CONTROL OF ANGULAR LEAF SPOT OF CUCUMBER[1]

A. B. Wiles and J. C. Walker

Angular leaf spot (*Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner) frequently becomes sporadic on cucumbers grown for processing in Wisconsin. Within individual fields it appears initially on a relatively small number of scattered plants. The rate of spread to surrounding plants and the severity of the disease are both extremely variable; a severe outbreak, developing suddenly in fields which previously had shown only a small number of infection centers, may be followed by almost complete disappearance of symptoms. At times the pathogen appears to spread slowly through a field and disease severity may be moderate for the remainder of the growing season. The seed supplied to the growers by processors usually is produced in the Rocky Mountain or Pacific Coast States, and is treated with either an organic mercury dust or a mercuric-chloride drench prior to delivery. The latter has long been a standard treatment for cucumber seed, but the dust is often used because of greater ease of application. After a severe outbreak of angular leaf spot in the summer of 1947 near Oconto, Wisconsin, where dust-treated seed had been used, it was recommended to the processors that the mercuric-chloride treatment be specified for all seed used in this area the next season. Field surveys in the summer of 1948 revealed that angular leaf spot was present, sometimes causing severe damage, in plantings of seed so treated in fields not known to have been previously planted to this crop.

This investigation was directed toward a study of 1) the effect of certain environmental factors, especially temperature and moisture, upon disease development; 2) the relation of infected seed to the initiation of epidemics; and 3) the relation of seed treatment to control.

RELATION OF ENVIRONMENT TO DISEASE DEVELOPMENT.—*Temperature relations.*—Carsner (1) observed that extremely hot summer weather did not appreciably check the development of angular leaf spot in the field. Jones and Doolittle (6) reported an outbreak of the disease in Wisconsin following a few days of warm, misty weather, and Weber (8) observed that seedling infection was less severe but not eliminated in dry weather with high temperatures. To determine more exactly the role of temperature in disease development, various controlled combinations of air and soil temperature were used in three series of experiments. In the first series the air and soil temperatures were approximately the same at each level; in the second the soil temperature was constant and the air temperature was varied; in the third the air temperature was constant and the soil temperature was varied. Each series consisted of three identical experiments. The plants were grown from seed planted in enameled pans in clean white silica sand and watered with distilled water. Approximately 7 days later, seedlings were transplanted, five per 6-in. glazed crock containing a mixture of three parts of greenhouse compost soil and one part of sand. The plants were inoculated by means of a light spraying with broth cultures of the organism from an atomizer-type sprayer, during the day at periods of high light intensity. A bell jar kept over each crock for 72 hr. maintained high humidity about the plants. Disease readings were taken at 5 and at 8 days. Control plants were treated in the same manner except that distilled water was used in place of the inoculum.

In the first series ten crocks each were kept at 16°, 20°, 24°, and 28° C. for 14 days; the plants were then inoculated and left at the same temperatures. Cucumber plants grown at 20° and 24° were large and succulent; those grown at 28° were rather spindly, and those grown at 16° were short and stunted. Disease development was most severe at the two highest temperatures. In the second series ten crocks each were kept at 16°, 20°, 24°, and 28° C. air temperature; Wisconsin soil temperature tanks were used to maintain a uniform soil temperature of 24° C. for 14 days; the plants were then inoculated and continued under the same environments. Under these conditions the host plants grown at 20° and 24° C. air temperature were large and succulent, those grown at 16° were short and stunted, and those grown at 28° were spindly. Disease development was most severe at the two highest air temperatures. In the third series, ten crocks were kept at an air temperature of 22° and Wisconsin soil temperature tanks were used to maintain soil temperatures of 24°, 28°, and 32° C. After 14 days the plants were inoculated and continued under the same conditions. Although the host plants grew reasonably well at all three of the soil temperatures, development was optimum at 24°. Plants growing at 32° C. soil temperature were most severely diseased; at 28° and 24° the symptoms were slightly less severe. When the pathogen was grown in nutrient broth the optimum temperature for most rapid growth was 24°, which agrees relatively closely with the report of Smith and Bryan (7).

The optimum temperatures for growth of the pathogen in pure culture, for host growth, and for disease expression were rather close. Air temperature of 20° to 24° C. plus soil temperature of about 24° C. were optimum for growth and development of the host plant. The optimum air temperature for disease development was slightly higher, i.e., 24° to 28°. Air and soil temperatures around 16° greatly retarded growth of the host as well as disease development. Soil temperature of 24 ° did not appreciably advance the growth of the host or the amount of disease where the air tempera-

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ture remained well below the optimum. However, soil temperature did affect both host growth and disease development where the air temperature was near the optimum and the temperature of the soil was appreciably higher than that of the air.

Moisture relations.—With certain bacterial plant pathogens it often has been difficult to obtain infection in the greenhouse unless host plants were subjected to high humidity. In their inoculations with *Pseudomonas lachrymans*, Smith and Bryan (7) subjected their plants to moist conditions for 30 hr. after inoculation with a suspension of the organism. In these investigations no macroscopic symptoms of the disease were obtained in the greenhouse, unless some form of moisture treatment was used; also, pre-inoculation moisture treatments resulted in no macroscopic symptoms except when followed by post-inoculation moisture treatments. Because of its role in the epidemiology of the disease, the minimum moisture treatment necessary for the expression of macroscopic disease symptoms was determined. Twenty crocks were kept at about 24° C. air and soil temperature for 14 days and then inoculated. Control plants were treated in the same manner except that distilled water was used in place of the inoculum. At intervals of 2, 4, 8, 12, 16, 20, 24, 48, 72, and 96 hr. two crocks containing inoculated plants and one containing control plants were removed from the moisture chamber. On plants having had the 4-hr. post-inoculation moisture treatment, small macroscopic lesions were observed at 5 days, but no lesions were observed at either 5 or 8 days on those having had the 2-hr. treatment. The other inoculated plants in the experiment had various degrees of disease development. Those plants which received the longest treatments had the largest lesions. Lesion size increased when plants with a short treatment were returned to the moisture chamber for additional treatment. This indicated that the length of the moisture treatment affected the size of lesions after penetration.

Although cucumber plants were readily infected in the greenhouse when given a post-inoculation moisture treatment, disease development under such conditions was rarely so severe as that often observed in the field. This feature also has been noted previously in connection with other bacterial plant diseases. In a study of wildfire of tobacco, Clayton (2) found that pre-inoculation water-soaking of leaves with a power sprayer produced disease symptoms as severe as those observed in the field. On leaves not receiving this pre-inoculation treatment, lesions were limited in size and were relatively inconspicuous. Johnson (5) reported that excessive intercellular water or water congestion predisposed plants to infection by certain microorganisms. In this investigation, leaves of 10 young cucumber plants in the four-leaf stage were lightly sprayed with water on their lower surfaces until they were water-soaked, care being taken not to injure the leaves. The plants were inoculated immediately and placed in a moist chamber at 24° C. Ten additional plants were given the same treatment but not inoculated, and 10 plants in a third group were inoculated but not water-soaked. All plants were placed in the same moisture chamber. After 72 hr. the plants which had received pre-inoculation water-soaking had severe disease development; those inoculated but not given pre-inoculation treatment had smaller and less conspicuous lesions; and those treated but not inoculated recovered from water-soaking.

Young cucumber plants in the four-leaf stage were water-congested artificially: the roots were washed until they were free from soil and water pressure was applied directly according to the method of Johnson (5). Check plants were handled in the same manner as in the previous experiment. After application of water pressure for about 15 min., visible water-congestion was observed first about the margins of the leaves and later about the larger veins. Each plant was treated for 30 min. prior to inoculation, then transferred to a flask of water and placed in a moisture chamber at 24° C. for 72 hr. Severe disease development invariably followed this treatment. Plants inoculated without the treatment had smaller and less conspicuous lesions; noninoculated plants recovered from congestion. When this experiment was repeated with variation in the degree of water congestion, the results showed that the higher the degree of water congestion the greater the extent of disease development. While plants remaining *in situ* in the soil were not visibly water-soaked, some congestion probably occurred, since periods as short as 4 hr. after inoculation, with plants under bell jars and within moisture chambers, increased the intercellular water content sufficiently to support infection, which in turn gave macroscopic symptoms of the disease. As the moisture treatment was lengthened the extent of disease development increased. These artificial treatments resulted in disease development most characteristic of that commonly observed in the field.

Relation of infected seed to initiation of epidemics.—In Wisconsin, cucumbers grown for processing are planted in rows 5–8 ft. apart. To facilitate picking, vines are trained to run lengthwise with the rows, leaving an open space 2–3 ft. wide between rows. The first symptoms of angular leaf spot usually are noted on the true leaves, seldom before the vines are in the third-leaf stage, but often after they begin to fruit. The plants affected at this stage usually are few and are scattered throughout the field. If environment is favorable, leaf lesions on plants in these primary centers enlarge rapidly and new lesions appear on near-by leaves. Subsequent spread of the pathogen is facilitated by spattering and wind-blown rain, by surface drainage water, and by pickers, as demonstrated by Carsner (1). The development of an epidemic is favored by warm weather, together with adequate moisture for penetration and for production of water congestion in the tissue.

In a previous paper (9) it was shown that lesions appear on the cotyledons of seedlings from infected seeds. No evidence was secured, however, of systemic invasion of the vascular elements of such plants. This

TABLE 1.—*Effect of various seed treatments on the germination of cucumber seeds from diseased fruits and upon the percentage of seedlings with infected cotyledons*

Series and treatment	Percentage of germination	Percentage of infected seedlings
A. None	95[a]	9.0[a]
1:1000 $HgCl_2$, 10 min.	96	5.0
B. None	95	8.5
1:1000 $HgCl_2$, 10 min.	96	4.5
1:1000 $HgCl_2$, 20 min.	97	3.5
1:1000 $HgCl_2$, 40 min.	96	3.0
C. None	93	9.0
1:1000 $HgCl_2$, 10 min.	95	4.0
Arasan dust	95	3.0
Water 50° C., 30 min.	91	4.5

[a]Each value is the average of results from four experiments.

would indicate that the inoculum in infected cotyledons must be disseminated externally from such parts to leaves of the same and near-by plants. In the summer of 1949 seed harvested the previous year from diseased fruits was planted extensively at Madison and at Oconto, Wisconsin, on soils not known to have been planted previously to cucumbers, and in all these plots leaf spot developed at some time during the growing season. At Oconto the disease appeared soon after the vines began to extend. At Madison, however, no signs of secondary infection appeared until late in the summer. These observations and others during 1947, 1948, and 1949 indicated that inoculum on primarily infected cotyledons remains viable as a source of secondary inoculum for a considerable time and in sufficient amount to instigate epidemics at any time during the season when temperature and moisture are favorable for dissemination and for water congestion of tissue.

RELATION OF SEED TREATMENTS TO CONTROL.—In extensive field trials with seed treatments for controlling angular leaf spot, Gardner and Gilbert (3, 4) were unable to obtain complete control but materially reduced the incidence of the disease by using the drench treatment with 1:1000 mercuric chloride. Weber reported complete control in Florida by this method. Because the degree of control obtained in Wisconsin fields with the mercuric-chloride treatment was variable, greenhouse studies on the relation of seed treatment to the control of angular leaf spot were made. Seed harvested from naturally infected fruits was planted in trays of clean sand and watered with distilled water. The treatments used, the percentage of germination after treatment, and the percentage of seedlings with infected cotyledons are in table 1. The results show that Arasan, hot water and mercuric chloride were about equally effective, but each treatment reduced the percentage of infected seedlings to only about one-half that from untreated seed. It is probable that the treatments destroyed bacteria on the surface of the seed and in the vicinity of the micropyle but did not affect those embedded within or beneath the seed coat. Although the number of infected seedlings from collections of infected seed was under 10 per cent, it was sufficient to induce epidemics when the same lots of seed were used in field tests. Moreover, the reduction of the percentage of infected seeds by one-half through treatment did not materially change the extent of epidemic development when the environment was favorable. It is doubtful, therefore, that any of the seed treatments used can be expected to have measurable beneficial effect on control of angular leaf spot.

DISCUSSION AND SUMMARY

The cotyledonary lesions, which are the source of primary inoculum in Wisconsin, develop when infected seed is germinated. Current seed treatment practices and others tried experimentally failed to eradicate the pathogen from naturally infected seed and reduced the percentage of infected seedlings only by approximately one-half. From relatively few infected plants scattered throughout a field the pathogen may build up to epidemic proportions by continual dissemination to surrounding healthy plants. The pattern of dissemination is irregular. Spattering and wind-blown rain and surface drainage water are effective agents; pickers and cultivation equipment are important means of spread when the vines are wet.

Environmental conditions after inoculation are important in determining the amount of disease development in the greenhouse. Experiments with various air and soil temperatures showed that when other environmental factors were favorable, the pathogen infected host plants over a fairly wide range of temperature. Disease severity increased with an increase in air and soil temperature up to 28° C. The optimum temperature for host development also was favorable for penetration and disease development. Moisture, however, is regarded as the most important environmental factor; no macroscopic symptoms were obtained in the greenhouse without some form of post-inoculation moisture treatment, and small macroscopic lesions increased in size as the moisture treatment was lengthened. Within limits, the longer the time of the post-inoculation moisture treatment the greater the size of the lesions and the more severe the disease. When the moisture content of the plants was increased before inoculation by means of water-soaking, water congestion, or in moist chambers and under bell jars, infection was more prompt and disease development more severe. In one series of the temperature studies in which 32° C. soil temperature and 22° C. air temperature were maintained, the plants were killed rapidly by the pathogen. In the same series less severe symptoms were obtained at 24° and 28° soil temperatures and with 22° air temperature. While extreme water congestion in cucumber plants *in situ* in the soil was not obtained, any treatment which tended to promote submacroscopic congestion aggravated the disease.

In the study of field development of angular leaf spot in Wisconsin in 1948, 1949, and 1950, water congestion was highly important in determining the rate and degree of epidemic development. Warm days followed by an extreme drop in temperature during the night

seemed to advance the disease most effectively after primary infection centers were established. These conditions provided not only night dews favorable to penetration, but high rates of water absorption in warm soil. Consequent high transpiration leads to greater water congestion when transpiration is checked suddenly by low night temperature.

It is doubtful that any seed treatment so far devised is sufficiently effective as an eradicant to be of real value in control. While the primary disease centers may be reduced in number by approximately one-half, the ultimate effect upon epidemic development would be negligible when favorable temperature and moisture prevail for dissemination of and penetration by the pathogen, and for water congestion of the host tissue. Until a method for complete eradication of the pathogen is devised, emphasis should be given to studying areas for cucumber seed production where climate effectively prevents seed contamination and infection.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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PHYTOPATHOLOGICAL NOTES

Transmission of Safflower Rust on Treated Seed. C. A. THOMAS. Approximately 100,000 acres of safflower, *Carthamus tinctorius* L., were grown in the United States in 1950. Rust, *Puccinia carthami* (Hutz.) Corda, was among the more important diseases affecting the crop.[1] This disease is probably unique among the rusts in that it is seed-borne. The fact that it may be transmitted by teliospores carried on the seed has been demonstrated by Prasada and Chothia.[2]

This note reports the dissemination of rust in 1950 on treated seed and the effectiveness of several seed treatments for its control.

Almost all of the seed for commercial plantings in 1950 was from rust-infested fields in western Nebraska, eastern Colorado, and eastern Wyoming. The seed was treated with Ceresan M (7.7 per cent ethyl mercury p-toluene sulfonanilide) at 0.5 oz. per bushel. The author observed many commercial fields and experimental plantings in 1950 in the above regions and in Kansas, Arizona, and California. Certain isolated fields planted with the treated seed of the variety N8 in Kansas, Colorado, and California, that had not been previously planted to safflower, showed light to heavy rust infection. It appeared that this infection probably came from seed-borne inoculum.

Tests were carried out in the greenhouse at Beltsville, Maryland, to determine the amount of rust transmitted by treated and untreated seed. Seed of the variety N8 from rust-infected plants at Scottsbluff, Nebraska, was supplied for these tests by Mr. Albert Hoffman of the University of Nebraska. Seeds were sown in flats of steamed soil and the seedlings were observed for rust infection over 4 to 8 weeks. Pycnia developed on the hypocotyls and cotyledons of check plants 10 to 12 days after planting and uredia, followed by telia, later developed in the same pustules.

Ceresan M was tested most extensively since it is the most effective material known for controlling a seed-borne *Alternaria* sp. that causes damping-off and leaf blight of safflower.[3] Other seed-treatment materials

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TABLE 1.—*Effect of seed treatment on transmission of safflower rust, Beltsville, Maryland, 1950*

Seed treatment		Number of seeds planted	Percentage of seedlings emerged—infected
Material	Rate per bu. (oz.)		
Check		3600	70.1—18.2
Arasan	½	1500	69.8— 1.24
Arasan	5	500	74.6— 0.268
Arasan	Excess	3800	73.4— 0.251
Dow 9B	Excess	400	68.7— 0.36
Seedox	Excess	400	66.0— 1.50
Panogen	1	500	71.4— 1.68
Panogen	2	500	72.6— 1.65
Ceresan M	½	1500	76.8— 1.47
Ceresan M	1	1500	75.1— 2.75
Ceresan M	2	3000	77.4— 1.51
Ceresan M	3	1500	73.3— 1.09
Ceresan M	5	1500	73.4— 0.54
Ceresan M	7	1500	73.8— 0.27
Ceresan M	Excess	8400	59.8— 0.019
Ceresan M	½[a]	1000	70.2— 1.42
Ceresan M	1[a]	1000	72.6— 1.79
Ceresan M	2[a]	1000	76.4— 1.31
Parzate	Excess	3000	71.7— 0.00
Phygon XL	Excess	3000	73.4— 0.00

[a] Jars completely filled with treated seed and sealed for 48 hr.

tested were Arasan (50 per cent tetramethyl thiuram-disulfide), Dow 9B (50 per cent zinc trichlorophenate), Seedox (trichlorophenylacetate), Panogen (2.1 per cent methyl mercury dicyan diamide), Parzate (65 per cent zinc ethylene bisdithiocarbamate), and Phygon XL (50 per cent dichloronaphthoquinone). All except Panogen were applied as dusts. In most cases treated seed was stored in open paper bags for one week before planting.

The results, in table 1, show that 18.2 per cent of the seedlings grown from untreated seed were affected with rust. All treatments appreciably reduced seedling infection and completely eliminated hypocotyl infection. Ceresan M at dosages of 0.5 to 9.0 oz. per bu., the maximum amount of dust the seed would hold, did not control rust completely, although at the highest dosage only one infected seedling was found in over 5,000 plants. The maximum dosage usually caused some reduction in stand. It thus appeared that the rust infection, observed in the fields mentioned above, resulted from inoculum carried on or in the seed.

Hypocotyl infections usually girdled the stems and killed the plants. Effective seed treatments should result in marked increases in stand by eliminating hypocotyl infections and reducing the number of cotyledon infections. It was hoped a treatment giving perfect control would be found. Such a treatment would be particularly useful in establishing rust-free plantings of safflower in new areas. Parzate and Phygon XL gave complete control without injury in a limited number of tests, and further work with these materials is planned.—Division of Tobacco, Medicinal, and Special Crops, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland.

Resistance to White Rust in Horseradish Seedlings.[1] R. W. HOUGAS,[2] G. H. RIEMAN,[3] AND G. W. STOKES.[3] White rust, induced by the fungus *Albugo candida* (Chev.) O. Kuntze, is one of the most destructive foliage diseases of the cultivated horseradish, *Armoracia rusticana* Gaertn., Mey., & Scherb. It causes heavy losses in the important horseradish-producing areas of the United States.[4] Practical methods for the control of this disease on a commercial basis have not been developed. Breeding and selection for resistance to the disease has been impossible because the Common horseradish is male-sterile and produces no seed.

The smooth-leafed (Bohemian) horseradish from southern Europe is resistant to the disease. Five strains of this horseradish, introduced into the United States about 1900,[5] were distributed to experiment stations and grown to a limited extent in several horseradish-producing areas. The Bohemian type proved to be inferior to the Common horseradish in root type and quality and has not found favor with the commercial grower. Certain Bohemian clones, however, do produce some functional pollen.

Incorporation of the white rust resistance of the Bohemian strains with the desirable horticultural characteristics of the Common horseradish through hybridization has been suggested as a practical measure in the control of this disease. Seed failure has heretofore been the barrier to such an approach. Weber[6] made matings between the Common and Bohemian varieties of horseradish and obtained plump and shrunken seeds. Five of the plump seeds proved to be viable. Further matings have been made and numerous viable seeds have been procured. More than 200 seedlings have been grown and tested for resistance to the white rust disease.

Test plants were grown from root cuttings of the seedlings. Plants at the three- to four-leaf stage were inoculated in a moist chamber with spores from infected leaves of the Common horseradish. Bohemian horseradish plants were similarly inoculated and used as controls. The inoculated plants were held in a saturated atmosphere for 4 hr. and retained for the remainder of the trial in a humid greenhouse at 65° to 70° F.

A wide range of susceptibility to the disease was evi-

[1]Paper No. 460 from the Department of Genetics, University of Wisconsin.

[2]Agent, Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[3]Professor of Genetics and Research Assistant, respectively, Wisconsin Agricultural Experiment Station.

[4]Kadow, K. J., and H. W. Anderson. A study of horseradish diseases and their control. Ill. Agr. Exp. Sta. Bul. 469: 531-583. 1940.

[5]U. S. Department of Agriculture Plant Introductions: 3117, 3778, 5589, 5761, 5837. 1899-1901.

[6]Weber, W. W. Seed production in horseradish. Jour. Hered. 40: 223-227. 1949.

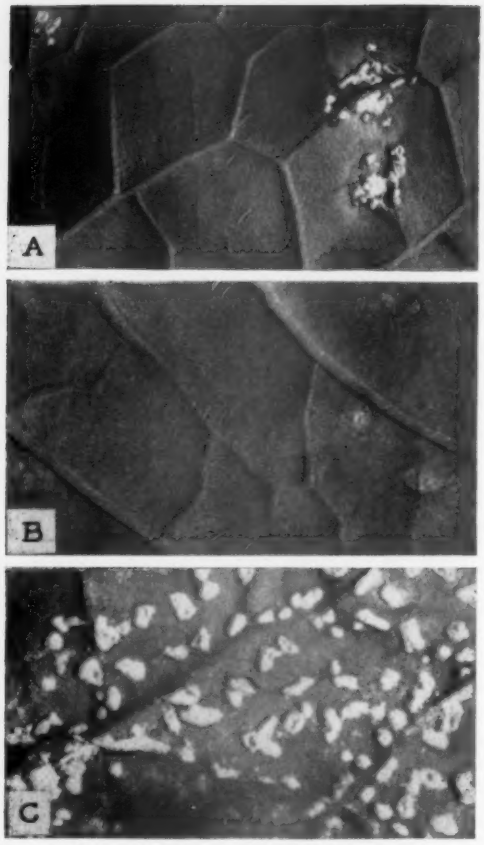

FIG. 1. Horseradish infected with the white rust disease. Leaf section, under surface, of: A, resistant Bohemian variety; B, highly resistant seedling; C, susceptible seedling with disease reaction similar to that of the Common variety.

dent in the seedling population. Three classes of host-parasite reaction were established:

A. Plants with resistance comparable to that expressed by the Bohemian variety. There was some sorus development (Fig. 1, A), and some sporulation under optimum conditions for the disease.

B. Plants with a higher degree of resistance to the disease than the Bohemian variety. Sorus development was limited (Fig. 1, B) and sporulation was not observed.

C. Plants which were more susceptible to the disease than the Bohemian variety. The variation within this class was large, ranging from plants with a slightly greater susceptibility to plants which appeared to be even more susceptible than the Common variety. Numerous sori developed (Fig. 1, C) and sporulation was abundant on the more susceptible seedlings.

Two hundred and seventeen seedlings, including 169 hybrids between the Common and Bohemian varieties and 48 selfs of the Bohemian variety, were inoculated. The distribution of the hybrid seedlings in classes A, B, and C was 4, 3, and 162, respectively; the distribution of the Bohemian selfs was 9, 8, and 31.

None of the seedling plants have been tested for white rust resistance in the field. The previous reports of Kadow and Anderson concerned with the high resistance of the Bohemian variety, and field observations confirming these reports would seem to indicate, however, that the conditions of these trials were more severe than those generally encountered in the field.—Division of Plant Exploration and Introduction, U. S. Department of Agriculture, and Wisconsin Agricultural Experiment Station.

Additional Notations on the Red Leaf Virus of Filaree.[1] CHRIS W. ANDERSON. The filaree red leaf virus was recently described by Frazier[2], while the writer reported[3] on the vector relationships between this virus and *Macrosiphum zerozalphum*. The present paper presents certain additional data concerning this virus disease.

The red leaf virus was recovered from all plant species reported susceptible by Frazier and from *Geranium molle*. *Zea mays* L., *Trifolium incarnatum* L., *Fragaria bracteata* Heller, *Pelargonium domesticum* Bailey, and several non-Geraniaceous species tested by Frazier showed no evidence of infection in tests by the writer. Tests with *Erodium chamaedryoides* and its variety *roseum* indicated that this plant is susceptible, but attempts to recover the virus failed.

Among the species reported by Frazier as showing no symptoms of infection is *Pelargonium hortorum*. In this one case, six of 38 test seedlings inoculated by the writer showed vague symptoms, unlike those of red leaf but possibly suggesting geranium leaf curl. Attempts to recover the virus from these plants failed, and *P. hortorum* still cannot be considered susceptible to the red leaf virus.

Frazier[2] listed the symptoms of red leaf infection, which include veinclearing, mild chlorosis, mild petiole curvature, stunting, leaflet curling, marginal reddening, and floral sterility. Perhaps the most characteristic symptoms of red leaf infection are a dark green veinbanding that can be seen from the abaxial side of leaves held against the light (Fig. 1, A), and marginal reddening, a symptom displayed by every suscept except *E. chamaedryoides*. Figure 1, C, illustrates the veinclearing and veinbanding that are the sole symptoms of red leaf infection in *E. chamaedryoides* and its variety *roseum*. Figure 2 shows the dwarfed, twisted fruits commonly produced during the early stages of infection by filaree plants inoculated in or near the flowering stage.

Evidence of infection was lacking in 147 white-stem filaree seedlings reared from such seed. Thus the virus

[1]A condensed portion of a thesis presented to the graduate division of the University of California, June 1950, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

[2]Frazier, Norman W. Red leaf, an aphid-borne virus disease of filaree. Phytopath. 41: 221-227. 1951.

[3]Anderson, Chris W. The insect vector relationships of the filaree red leaf virus, with special reference to a latent-period difference between nymphs and adults of Macrosiphum geranicola. Phytopath. 41: 699-708. 1951.

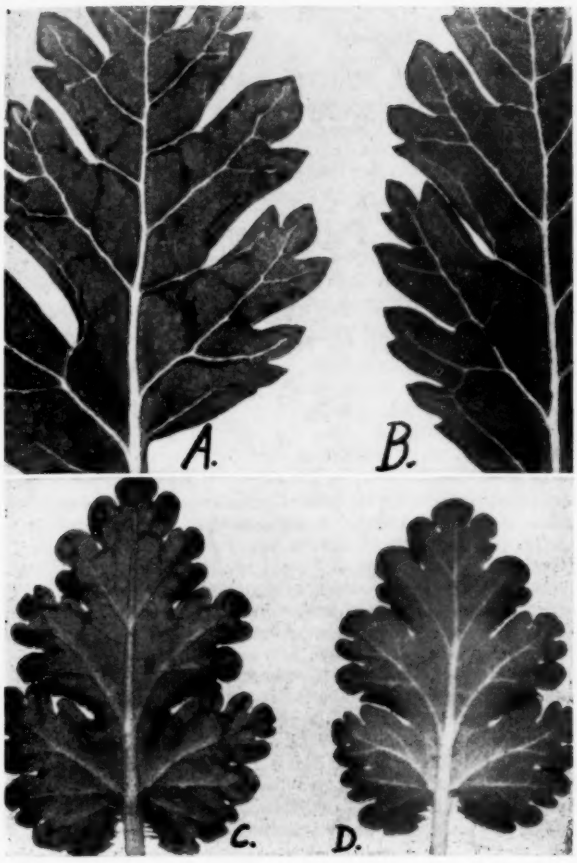

FIG. 1. Symptoms of the red leaf virus. A, veinbanding on leaflet of *Erodium moschatum* as seen against the light. B, healthy control. C, veinbanding on leaf of *E. chamaedryoides roseum*. D, healthy control.

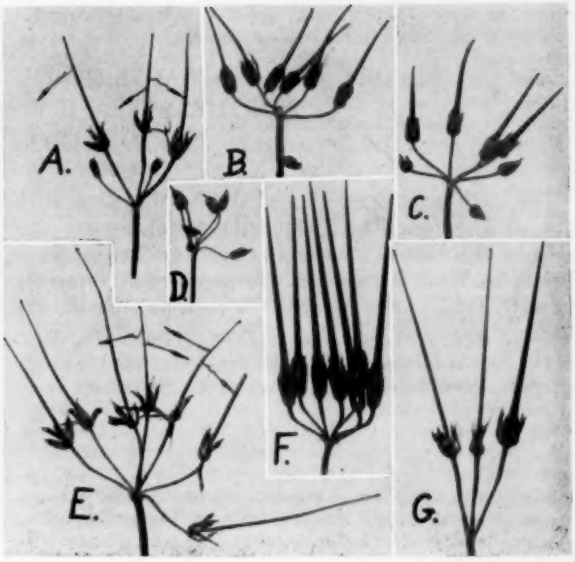

FIG. 2. Effects of the red leaf virus on the fruiting of *Erodium moschatum*. A–E, diseased; F–G, healthy. Note tendency (A, E) of carpels to remain attached to receptacles by their styles; dwarfed, twisted fruits (B, C); and sterile fruits produced by plants that have been diseased longer (D). Healthy fruits (F) are still green.

is rarely or never seed-borne, although it is possible that it might enter or remain in occasional seeds produced by plants during their late stages of infection.

Erodium moschatum is susceptible to curly top[4], California aster yellows[5], and western cucumber mosaic. *E. cicutarium* and *E. botrys* are also susceptible to the above three viruses [4, 5, 6, 7] while *E. cicutarium* and *E. cygnorum* are infected by tomato virescence[8] and *E. macrophyllum* is susceptible to curly top.[7] More recently, Freitag[9] has reported that *Erodium cicutarium* is susceptible to the Pierce's disease virus. Frazier[2] has differentiated the symptoms of red leaf from those of California aster yellows on white-stem filaree. The writer found that both curly top and western cucumber mosaic can be distinguished from red leaf on white-stem filaree primarily because these viruses do not cause the veinbanding or the striking marginal reddening of red leaf. Moreover, the margins of the young leaves of curly-top plants curl inward whereas those of red leaf curl outward. Western cucumber mosaic causes no fruit symptoms. Tomato virescence was not available, but Hill[8] seems to imply that virescence, which is not caused by red leaf, is a symptom of this virus on *E. cicutarium*. Limited tests indicated that tobacco mosaic and spotted wilt do not produce symptoms on *E. moschatum*.

The fact that the red leaf virus persists in its aphid vectors and is not juice-transmissible separates it from most other plant viruses. Host range and vector differences separate it from other persistent aphid-borne viruses. Thus, unless the red leaf virus is, as seems unlikely, a synonym of geranium leaf curl or of geranium mosaic, it is probably distinct from all other known viruses.—Agricultural Experiment Station, Gainesville, Florida.

[4]Carsner, E. Susceptibility of various plants to curly top of sugar beet. Phytopath. 9: 413-421. 1919.

[5]Frazier, Norman W., and Henry H. P. Severin. Weed-host range of California aster yellows. Hilgardia 16: 619-650. 1945.

[6]Mitchell, J. H. Weed host range of western cucumber mosaic. Unpublished M. A. Thesis. Univ. California (Berkeley). 1939.

[7]Severin, Henry H. P. Weed host range and overwintering of curly-top virus. Hilgardia 8: 261-280. 1934.

[8]Hill. A. V. Insect transmission and host plants of virescence (big bud of tomato). Austr. Counc. Sci. Ind. Res. Jour. 16: 85-90. 1943.

[9]Freitag, J. H. Host range of the Pierce's disease virus of grapes as determined by insect transmission. Phytopath. 41: 920-934. 1951.

REPORT AND ABSTRACTS OF THE SIXTH ANNUAL MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The sixth annual meeting of the Northeastern Division of The American Phytopathological Society was held in the Eastern States Farmers' Exchange Auditorium at West Springfield, Massachusetts, November 6 and 7, 1951. Approximately 80 persons attended the meeting representing Maine, New Hampshire, New York, Rhode Island, Massachusetts, Connecticut, Pennsylvania, New Jersey, and Maryland. Seventeen technical papers were presented. At the evening meeting, November 6, Dr. L. O. Kunkel spoke on "A review of forty years of research in the field of plant viruses."

Officers elected to serve during 1952 were as follows: *President*, J. M. HAMILTON; *Vice President*, S. G. YOUNKIN; *Secretary-Treasurer*, J. S. TIDD; *Councilor*, L. M. BLACK.

S. G. YOUNKIN
Secretary-Treasurer

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

The effect of fluorine on vegetation when applied through the substrate or in the atmosphere as influenced by plant nutrition and environmental factors. DAINES, ROBERT H., IDA LEONE, AND EILEEN BRENNAN. From studies at the New Jersey Agricultural Experiment Station to determine the effects of fluorine on vegetation it has been found that plants absorb fluorine both from the atmosphere and from the soil, thereby accumulating abnormally high fluorine contents often resulting in the development of typical symptoms of F toxicity. Different species vary in the minimum concentration of F necessary to produce visible injury, in their capacity for F uptake, and in the relation between F content and extent of injury. In experiments with tomato, plants in an optimum growing condition with respect to nitrogen, calcium, and phosphorus tend to be more susceptible to fluorine injury from the soil and, to a lesser extent, from the atmosphere. In soil studies as the pH of the soil was increased by the addition of lime, the degree of fluorine toxicity and the amount of fluorine absorbed by the plants were minimized. Moisture was of considerable importance in determining the extent of plant injury from atmospheric fluorine. Conditions conducive to greater degree of injury and fluorine absorption are high atmospheric humidity, turgidity of the plant, and wetting of the plant surfaces. The application of lime spray materials to plants prior to fluorine fumigation increases the fluorine content of the leaf. Since much of this is removable by washing with water or detergents, it is believed that the presence of calcium on the leaf surface actually serves as a deterrent to fluorine absorption by the leaves, much as it decreases root absorption in the soil. The problem of distinguishing between fluorine coming from the atmosphere or from the substrate is partly solved by analyses of leaves and roots, inasmuch as the distribution gradient is characteristic of the source of entry. Atmospheric fluorine results in a high leaf and low root content; soil fluorine causes a high leaf and even higher root content. Furthermore, work in progress indicates that a considerable amount of fluorine in fumigated foliage is removable by washing with water or detergents. This coincides with the amount normally dissipated by a fumigated plant within several weeks after fumigation. To date it does not appear that there is a comparable loss of fluorine from root-treated plants.

Effect of railroad transit conditions on certain potato tuber diseases. FOLSOM, DONALD, HARRY Q. ROACH, JAMES S. WIANT, AND J. KAUFMAN. Ten samples from each of various potato stocks, varying in weight from 10 to 100 lb., were examined in Maine. The same person examined the same number of duplicate samples after shipment to New York City. The transit period was about 1 week and the mean car temperature during shipment was 43° to 50° F. Usually leafroll net necrosis and stem-end browning (virus) increased in severity in transit; mahogany browning (induced by several months' exposure to low temperatures next to the outside walls of storage bins) and bacterial ring rot increased in prevalence and severity; late blight rot did not increase after mid-November, but bacterial soft rot and Fusarium rot associated with late blight rot did increase. When stocks soiled by breakdown of frozen tubers had been "cleaned" with rubber brushes and sawdust, scorable bruises, lesions, and soft rot increased in transit. Mahogany browning occurred in all parts of the tuber, but it was most prevalent and severe in the larger tubers of all stocks.

Soft rot of lettuce and escarole caused by Pseudomonas marginalis. FRIEDMAN, B. A. A number of isolations were made from leaves of Arizona-grown head lettuce (*Lactuca sativa* L.) and Florida-grown escarole (*Cichorium endivia* L.) found to have a bacterial slimy soft rot upon examination at the New York market. Three pathogenic isolates were obtained from escarole and six from lettuce. All nine isolated produced soft rot upon leaf inoculation of both lettuce and escarole. The bacterial isolates consisted of Gram negative rods. They formed a greenish fluorescent pigment in asparagine broth but no pyocyanin; liquefied gelatin; reduced nitrates to nitrites; formed acid, but no gas, in brom cresol purple synthetic broth from sucrose, lactose, and maltose; and failed to form acid from salicin. The bacteria were considered to be identical with *Pseudomonas marginalis* (Brown) Stevens.

Breeding carnations for resistance to Fusarium wilt. GUBA, E. F. The performance of numerous carnation varieties to Fusarium wilt caused by the fungus *F. dianthi* Prill. & Delacr. was determined by surveys, questionnaires, and artificial infection studies. Selfing and crossing flowers of resistant types, and testing the reaction of the progeny with inoculum of the fungus from pure culture, have yielded numerous resistant seedlings in a variety of colors and types. The most promising seedlings are assigned to growers for further appraisal and commercial development. By this procedure several new varieties resistant to Fusarium wilt originated at Waltham have been introduced to the trade. These are: Waltham Pink (John Briry × Dorothy Napier); Regal Pink (Puritan × Helen Hussey); Spicy Rose (Puritan × Eleanor); and Mrs. E. F. Guba, almond and pink variegated (John Briry × Dorothy Napier). These four varieties are also resistant to rust (*Uromyces caryophyllinus* (Schr.) Wint.) and blight (*Alternaria dianthi* Stev. & Hall). Other promising wilt-resistant seedlings in the process of appraisal and development are: No. 7, Yellow variegated (Elizabeth Rowe × Helen Hussey); No. 10, Rose Pink (parentage unknown); No. 23, Orange Crimson variegated (John Briry × Helen Hussey); No. 23-W, Light Rose (Elizabeth Rowe × Helen Hussey); No. 40, Rose Pink (Elizabeth Rowe × Helen Hussey);

No. 61, Yellow Crimson variegated (Puritan × Helen Hussey); No. 209, Dark Pink (John Briry × Woburn); and No. 210, Light Pink (John Briry × Eleanor).

Inherited resistance to Colletotrichum phomoides in tomato. HOADLEY, A. D. Hereditary anthracnose resistance of a relatively high order is present in some lines of the cherry tomato, *Lycopersicon esculentum* var. *cerasiforme*. Anthracnose-resistant, large-fruited tomato lines have been developed through repeated backcrosses of resistant lines selected from a cross between the resistant cherry tomato and the susceptible Rutgers variety. Single plant selections were made with detached fruit artificially inoculated with a spore suspension of *Colletotrichum phomoides*. The relative resistance of large-fruited lines was determined through replicated tests of naturally infected fruit. In one test, the percentage of anthracnose averaged 32.5 for Rutgers, 7.8 to 16.3 for the resistant selections, and 0.5 for the cherry tomato. Fruit size of the resistant selections varied from 126 to 192 fruits per 35-lb. basket as compared to 123 for Rutgers. The inheritance pattern of anthracnose resistance has not been determined but is apparently of a complex nature.

Use of primary lesions to determine relative susceptibility of individual plants. HOLMES, FRANCIS O. Primary-lesion methods are now applicable to measurement of concentrations of some fourteen phytopathogenic viruses. For this purpose, test plants are chosen to be as uniformly susceptible as possible and variations in lesion counts are interpreted as indicating differences in viral concentrations. By reversing this process and using inoculum of known infectivity, variations in lesion counts can be utilized to indicate differences in susceptibility among individual plants. Sometimes, however, it is desired to test susceptibilities of plants that do not ordinarily show primary lesions. Some change in technique is then required. This is illustrated in recent studies of tomato plants derived from hybrids of low susceptibility to infection by tobacco-mosaic virus. Ordinary strains of the virus do not induce formation of visible primary lesions in foliage of tomato. Accurate separation of plants of high from those of low susceptibility was attained in this case by substituting for ordinary tobacco-mosaic virus a mutant strain, designated as 1952D, that produces necrotic primary lesions at sites of inoculation in tomato leaves whenever infection occurs. The number of necrotic lesions depends on, and serves to measure, the relative susceptibility of individual plants to infection by this and ordinary strains of the virus.

Relation of laboratory ED50 to field performance of fungicides. KEIL, HARRY L., JAMES G. HORSFALL, AND SAUL RICH. Earlier it was reported (Chapman et al., Phytopath. 40: 4-5. 1950) that given equal ED50 values, organic fungicides, in general, perform in the field in the order of their resistance to rainfall. Resistance to rainfall was measured by the slope of the wash-off curve. During the past three years, a number of miscellaneous organic compounds have been field-tested as protectants against apple scab, bean anthracnose, and other diseases. These fungitoxic compounds were all chosen because of their high resistance to removal by rain; i.e., they gave wash-off curves with flat slopes. In general, the field performance of these materials was directly related to their laboratory ED50 values. The ED50 values for fungitoxicity were obtained from dosage-response data using the spores of *Stemphylium sarcinaeforme* and *Sclerotinia fructicola*. Of the two organisms, *S. fructicola* appeared to be more efficient for finding protectants against apple scab, whereas *S. sarcinaeforme* seemed better for finding protectants against bean anthracnose. Some compounds which showed unexpectedly poor field performance were afterwards found to be chemically unstable in a thin spray deposit. It should be emphasized that there is considerable variability in the relation of laboratory evaluations to field performance.

Root injury as a factor in the assessment of chemotherapeutants for Fusarium wilt. KEYWORTH, W. G., AND A. E. DIMOND. Certain types of injury to the roots of tomato plants before their inoculation with *Fusarium lycopersici* resulted in greatly reduced disease severity. Such injury was accomplished in three ways: (a) dipping the roots in water at 55° C. for 30 seconds, (b) periodic root-pruning, and (c) the application of sublethal doses of aluminum, calcium, ferric, potassium, and sodium chlorides, sodium sulfate, potassium bromide, and hydrogen peroxide. The various treatments used had only one factor in common, viz., root injury. It therefore appears that disease reduction resulted from the root injury *per se* and not from the action of any specific treatment. In further tests with hydrogen peroxide the roots of treated and check plants were cut to ⅜ in. long immediately before inoculation. The treated plants still showed little disease although the checks were severely wilted. It is thus probable that the effect resulted from a change in host resistance and not merely from inhibition of the pathogen at the injured root surfaces. Some, but not all, of the chemicals selected by the tomato Fusarium wilt chemotherapy test cause considerable root injury (e.g., n-octadecyltrimethylammonium pentachlorophenate—Code No. HD3). Such injury must therefore be taken into account in considering the mode of action of these chemicals.

Temperature in relation to retention of viability of encysted larvae of the golden nematode of potato, Heterodera rostochiensis Wollenweber. MAI, W. F. Golden nematodes were stored air-dry in Petri dishes and in moist soil for a 6-month period at six temperatures ranging from 3° to 37° C. Survival of encysted larvae in moist soil decreased as the temperature increased, but, with the exception of a higher mortality at 37°, the storage temperature had very little effect upon the retention of viability of air-dried encysted larvae. The average soil temperatures at a depth of 6 in. for Long Island, New York, and for Leesburg, Florida, during the 8-month period when potato crops are not grown commercially in each area were duplicated in soil temperature tanks. The average monthly New York soil temperatures varied from 1° to 23° C. with an average of 9°, and the Florida monthly soil temperatures varied from 21° to 30° C. with an average of 26°. The temperatures were changed at the beginning of each month to correspond with the average monthly temperature in the area that the particular tank was to represent. A high percentage of encysted larvae remained viable after exposure to Florida soil temperatures for an 8-month period. The survival of nematodes, however, at Florida temperatures was significantly lower than the survival at New York soil temperatures.

Inability of aster-yellows and corn-stunt viruses to multiply in each other's vectors. MARAMOROSCH, KARL. An attempt was made to determine whether or not the aster yellows virus can multiply in the vector of corn stunt, *Dalbulus maidis*, and conversely, whether the corn stunt virus can multiply in the vector of aster yellows, *Macrosteles divisus*. The viruses were obtained from infective insects macerated at 0° C. The insect juices were diluted to 10^{-2} with neutral buffered 0.25 M NaCl solution and centrifuged at 3000 r.p.m. for 5 min. The supernatant fluids were used for insect injections. Three "blind" passages were made at 30-day intervals. Aster leafhoppers with corn stunt virus were kept on asters at 30° C. and corn leafhoppers with aster yellows virus on corn at 25° C. Finally the juices of both groups of surviving inoculated insects were used for the injection of the proper vector species and the leafhoppers were individually tested on susceptible plants for the remainder of their lives. No transmission occurred in either group. Controls proved virus-free. It was concluded, therefore, that aster yellows and corn stunt viruses do not multiply in each other's vectors. The ability of aster yellows virus to multiply only in its own vector may explain its specificity in transmitting.

Pruning elms affected with Dutch elm disease. MARSDEN, DAVID H. Small-scale trials have shown that the

elimination of Dutch elm disease from individual trees by pruning is possible in a surprisingly high percentage of selected cases. All trees involved were inoculated naturally with *Ceratostomella ulmi* (Schwarz) Buisman. The diseased trees were detected during the course of routine sampling of elms for disease confirmation in Amherst, Massachusetts, and vicinity. In 1950, by prompt pruning out of all visibly affected parts, 8 of 12 trees were apparently freed of the disease. In 1951 twelve additional trees were pruned, of which seven manifested no further symptoms during the season. Early detection of wilt symptoms and prompt removal of all symptomatic parts are essential to success. More than 5 per cent wilting in the crown and discoloration of sapwood extending into the trunk militate against the probable success of pruning.

A method of obtaining a high incidence of powdery mildew on snap beans in the greenhouse for fungicide screening tests. Miller, Harold J. Powdery mildew readily occurs on snap beans in the experimental greenhouse, but growth and maturity of the bean plant with subsequent defoliation exceeds the rate of spread of this disease so that adequate counts are difficult to obtain in fungicide tests. Treatment of plants at primary leaf stage by a spray application of maleic hydrazide (0.125 per cent) or pentachlorobenzoic acid (0.01 per cent) inhibits growth of trifoliate leaves and causes the primary leaves to be retained, with a resulting increase in vigor. Primary leaves can be sprayed once with fungicide and kept in the greenhouse until mildew builds up sufficiently for a good reading. Typical data on control as indicated by average number of spots on two leaves of four plants are as follows: check (maleic hydrazide), 118; check (pentachlorobenzoic), 210; wettable sulfur (4-100), 0.0; NP-770 (a complex phenol) (1-100), 8.0.

Retention of strains of tobacco mosaic virus in tomato seeds. Raychaudhuri, S. P. Diseased tomatoes showing symptoms of internal browning were collected from New Brunswick and Bridgeton, New Jersey. Seeds obtained from these tomatoes were germinated in the greenhouse at weekly intervals. Extracts from seedlings were tested on *Nicotiana glutinosa* to determine the presence of tobacco mosaic virus. Only seedlings raised from seeds stored for one week were found infected with the strains of virus associated with internal browning. Stored seeds from tomatoes infected with the ordinary strain of tobacco mosaic virus, however, retained the virus for 27 days. Dry seeds from tomatoes affected by internal browning disease retained the virus externally for at least 2 weeks, although the amount of virus was much reduced.

Using half-tree plots for increasing efficiency of fungicide tests. Rich, Saul. Making comparisons on the two opposite halves of the same biological unit has been successfully employed in the half-leaf technique used in virus studies. The basic premise is that two halves of the same biological unit are much less heterogeneous in their response than are two separate individuals. This approach was tried in comparing fungicides in the field by the use of half-tree plots. Different treatments were applied to opposite halves of the same tree. This technique had the added advantage of doubling the number of treatments that could be tested. Half-tree plots were used on 28 standard McIntosh trees for the past two years. The treatments were applied to the east and west halves of the trees. An analysis of variance segregated the block error, and showed no significant east-west effect. Other regression studies disclosed that the amount of disease in poor treatments was directly affected by the amount of disease in the opposite half-tree. Good treatments, however, were not affected in this manner. There was no detectable cross-tree contamination by spray drift. This technique can perhaps be used to detect systemic effects.

Bacterial fire blight of raspberry. Starr, Mortimer P., Canuto Cardona, and Donald Folsom. From diseased Latham red raspberry in central Maine, where apple fire blight is rare and has not been found since 1943, a bacterial organism was isolated which bacteriologically was identical with *Erwinia amylovora.* Midwinter greenhouse inoculations and reisolations in Maine repeatedly proved the organism pathogenic to raspberry. Contemporary inoculations showed the organism to be nonpathogenic to apple and showed that cultures of *Erwinia amylovora* from apple were pathogenic to apple but not to raspberry. The name *Erwinia amylovora* f. sp. *rubi* has been suggested for the raspberry organism (Phytopath. 41: 919. 1951). Natural field infection and greenhouse infection of raspberry occurred on leaves, flower buds, and stems, caused a purple discoloration, were soon followed by exudation of bacterial drops, and finally were followed by invasion of diseased tissues by *Fusarium, Botrytis, Alternaria,* and *Cladosporium.* Field infection was apparently much reduced in 1951 by prompt removal of all young purple shoots.

Awl nematode injury on Chinese waterchestnuts. Tarjan, A. C. Since the successful introduction of Chinese waterchestnut (*Eleocharis dulcia*) into the United States a number of years ago, interest in the culture of this plant has been increasing. In one extensive planting in Southeastern United States symptoms of decline of these plants have recently been noticed. Examinations of roots and soil from affected plants revealed the presence of awl nematodes, *Dolichodorus heterocephalus.* In an attempt to evaluate the effect of these nematodes on waterchestnuts, two lots of 12 replicate 1-gal. crocks each containing three plants growing in a water-saturated sandy loam were inoculated with nematode suspensions containing either 750 or 1500 *D. heterocephalus.* The third lot of 12 crocks remained uninoculated. Crocks were completely randomized and plants grown at an average soil temperature of 24° C. After 3½ months, data were obtained on total weight of plants, weight of roots, and number and weight of corms produced. Noninoculated control plants had a significantly higher total and root weight than inoculated plants. Counts of *D. heterocephalus* from the soil in which plants had been growing indicated that these nematodes found *E. dulcis* a suitable host and had reproduced on it.

Control of gray leaf spot and anthracnose of tomato. Younkin, S. G., F. L. Merwarth, and A. D. Hoadley. Gray leaf spot of tomatoes caused by *Stemphylium solani* occurred in epiphytotic proportions in New Jersey, Pennsylvania, and on the Eastern Shore of Maryland and Virginia in 1949, 1950, and 1951. Tests were conducted during two years to determine efficacy of different fungicides on control of gray leaf spot and anthracnose. In one typical test defoliation averaged 15, 34, 41, 56, and 75 per cent in replicated plots sprayed seven times with manganese ethylene bisdithiocarbamate, zineb, ziram, tribasic copper sulfate, and no fungicide, respectively. Incidence of anthracnose (*Colletotrichum phomoides*) in three tests showed that manganese ethylene bisdithiocarbamate was equal to ziram in controlling anthracnose. Where defoliation was prevalent zineb and ziram controlled anthracnose equally well, but in the absence of pronounced defoliation ziram was more effective than zineb.